pK_a measurements for the SAMPL6 prediction challenge for a set of kinase inhibitor-like fragments

Mehtap Işık[1,2] , Dorothy Levorse[3] , Ariën S. Rustenburg[1,4] , Ikenna E. Ndukwe[5] , Heather Wang[6] , Xiao Wang[5] , Mikhail Reibarkh[5] , Gary E. Martin[5] , Alexey A. Makarov[6], David L. Mobley[7] , Timothy Rhodes[3*] , John D. Chodera[1*,6]

[1] Computational and Systems Biology Program, Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, New York, NY 10065, United States
[2] Tri-Institutional PhD Program in Chemical Biology, Weill Cornell Graduate School of Medical Sciences, Cornell University, New York, NY 10065, United States
[3] Pharmaceutical Sciences, MRL, Merck & Co., Inc., 126 East Lincoln Avenue, Rahway, New Jersey 07065, United States
[4] Graduate Program in Physiology, Biophysics, and Systems Biology, Weill Cornell Medical College, New York, NY 10065, United States
[5] Process and Analytical Research and Development, Merck & Co., Inc., Rahway, NJ 07065, United States
[6] Analytical Research and Development, Merck & Co., Inc., Rahway, NJ 07065, United States
[7] Department of Pharmaceutical Sciences and Department of Chemistry, University of California, Irvine, Irvine, California 92697, United States

*For correspondence:
john.chodera@choderalab.org (JDC)
timothy_rhodes@merck.com (TR)

SUPPLEMENTARY INFORMATION

Table SI 1. Procurement details of SAMPL6 pK_a challenge compounds

SAMPL6 Molecule ID	Group	Supplier	LOT	CAT	Supplier reported purity	CAS	eMolecules ID	canonical isomeric SMILES	Experimental Molecule ID
SM01	fragment-like	AchemBlock	11549	10222	95%	521937-07-05	6679830	c1cc2c(cc1O)c3c(o2)C(=O)NCCC3	M01
SM02	fragment-like	ChemDiv	CM02432403	3232-0333			1327907	c1ccc2c(c1)c(ncn2)Nc3cccc(c3)C(F)(F)F	M02
SM03	fragment-like	ChemDiv		Z27474679			1228629	c1ccc(cc1)Cc2nnc(s2)NC(=O)c3cccs3	M03
SM04	fragment-like	ChemDiv		Z126957826			30719859	c1ccc2c(c1)c(ncn2)NCc3ccc(cc3)Cl	M04
SM05	fragment-like	ChemDiv		Z119335440			18908671	c1ccc(c(c1)NC(=O)c2ccc(o2)Cl)N3CCCCC3	M05
SM06	fragment-like	ChemDiv		Z28487401			18893169	c1cc2cccnc2c(c1)NC(=O)c3cc(cnc3)Br	M06
SM07	fragment-like	Enamine	2017-0168841	Z57161635	95%	100818-54-0	1327878	c1ccc(cc1)CNc2c3ccccc3ncn2	M07
SM08	fragment-like	Enamine	2017-0168838	Z57157353	95%	65418-08-8	1367649	Cc1ccc2c(c1)c(c(c(=O)[nH]2)CC(=O)O)c3ccccc3	M08
SM09	fragment-like	Enamine	2017-0168839	Z220564816	95%		1865544	COc1cccc(c1)Nc2c3ccccc3ncn2.Cl	M09
SM10	fragment-like	Enamine	2017-0168843	Z69130143	95%	35056-22-5	23354217	c1ccc(cc1)C(=O)NCC(=O)Nc2nc3ccccc3s2	M10
SM11	fragment-like	Maybridge	142989	RJC00689SC	90%	5334-30-5	719540	c1ccc(cc1)n2c3c(cn2)c(ncn3)N	M11
SM12	fragment-like	Maybridge	265423	DP00818SC			1859493	c1ccc2c(c1)c(ncn2)Nc3cccc(c3)Cl.Cl	M12
SM13	fragment-like	Maybridge	248841	GK03474SC			5828805	Cc1cccc(c1)Nc2c3cc(c(cc3ncn2)OC)OC	M13
SM14	fragment-like	Enamine		Z57290870			31653344	c1ccc(cc1)n2cnc3c2ccc(c3)N	M15
SM15	fragment-like	Enamine		Z1318268952			37095168	c1ccc2c(c1)ncn2c3ccc(cc3)O	M16
SM16	fragment-like	VitaScreen		STK098832			1284691	c1cc(c(c(c1)Cl)C(=O)Nc2ccncc2)Cl	M18
SM17	fragment-like	VitaScreen		STK032731			1444229	c1ccc(cc1)CSc2nnc(o2)c3ccncc3	M19
SM18	drug-like	Enamine		Z278071350			18897105	c1ccc2c(c1)c(=O)[nH]c(n2)CCC(=O)Nc3ncc(s3)Cc4ccc(c(c4)F)F	D01
SM19	drug-like	Enamine		Z30206127			3365457	CCOc1ccc2c(c1)sc(n2)NC(=O)Cc3ccc(c(c3)Cl)Cl	D02
SM20	drug-like	VitaScreen		STL282831			46568819	c1cc(cc(c1)OCc2ccc(cc2Cl)Cl)/C=C/3\C(=O)NC(=O)S3	D05
SM21	drug-like	VitaScreen		STL368658			1574612	c1cc(cc(c1)Br)Nc2c(cnc(n2)Nc3cccc(c3)Br)F	D06
SM22	drug-like	VitaScreen		STK070581			536848	c1cc2c(cc(c(c2nc1)O)I)I	D07
SM23	drug-like	VitaScreen		STK097966			4375254	CCOC(=O)c1ccc(cc1)Nc2cc(nc(n2)Nc3ccc(cc3)C(=O)OCC)C	D08
SM24	drug-like	VitaScreen		STK090644			1415746	COc1ccc(cc1)c2c3c(ncnc3oc2c4ccc(cc4)OC)NCCO	D09

Table SI 2. Calculated properties and descriptors of compounds of 28 compounds selected and procured for pKa challenge. pKa measurement experiments were successful 24 molecules labeled SM01-SM24.

SAMPL6 Molecule ID	Experimental Molecule ID	group	Epik pK_as in [3,11] range	OpenEye XlogP	Molecular Weight (g/mol)	eMolecules reported availability (mg)	Number of rotatable bonds	Number of UV-chr. units	eMolecules ID	canonical isomeric SMILES
SM01	M01	fragment-like	[9.119]	3.27	289.26	184	0	27	6679830	c1cc2c(cc1O)c3c(o2)C(=O)NCCC3
SM02	M02	fragment-like	[4.05]	3.61	301.39	101	3	36	1327907	c1ccc2c(c1)c(ncn2)Nc3cccc(c3)C(F)(F)F
SM03	M03	fragment-like	[7.12]	3.52	269.73	379	5	14	1228629	c1ccc(cc1)Cc2nnc(s2)NC(=O)c3cccs3
SM04	M04	fragment-like	[5.564]	3.79	304.77	415.5	3	36	30719859	c1ccc2c(c1)c(ncn2)NCc3ccc(cc3)Cl
SM05	M05	fragment-like	[5.346]	2.92	328.16	424.3	4	18	18908671	c1ccc(c(c1)NC(=O)c2ccc(o2)Cl)N3CCCCC3
SM06	M06	fragment-like	[4.001, 10.328]	2.91	235.28	406	3	37	18893169	c1cc2cccnc2c(c1)NC(=O)c3cc(cnc3)Br
SM07	M07	fragment-like	[5.564]	2.80	293.32	208.7	3	36	1327878	c1ccc(cc1)CNc2c3ccccc3ncn2
SM08	M08	fragment-like	[4.109]	3.14	287.74	232.1	3	59	1367649	Cc1ccc2c(c1)c(c(c(=O)[nH]2)CC(=O)O)c3ccccc3
SM09	M09	fragment-like	[4.05]	2.72	311.36	119.7	3	36	1865544	COc1cccc(c1)Nc2c3ccccc3ncn2.Cl
SM10	M10	fragment-like	[8.672]	1.50	211.22	149.1	6	28	23354217	c1ccc(cc1)C(=O)NCC(=O)Nc2nc3ccccc3s2
SM11	M11	fragment-like	[3.869]	3.90	292.16	3430	1	31	719540	c1ccc(cc1)n2c3c(cn2)c(ncn3)N
SM12	M12	fragment-like	[4.05]	2.60	295.34	7366	2	36	1859493	c1ccc2c(c1)c(ncn2)Nc3cccc(c3)Cl.Cl
SM13	M13	fragment-like	[4.267]	2.91	235.28	1864	4	36	5828805	Cc1cccc(c1)Nc2c3cc(c(cc3ncn2)OC)OC
-	M14	fragment-like	[5.564]	2.33	209.25	208.7	3	36	1327878	c1ccc(cc1)CNc2c3ccccc3ncn2
SM14	M15	fragment-like	[6.348]	2.22	210.23	50213	1	40	31653344	c1ccc(cc1)n2cnc3c2ccc(c3)N
SM15	M16	fragment-like	[5.82, 8.709]	4.23	263.33	21650.2	1	40	37095168	c1ccc2c(c1)ncn2c3ccc(cc3)O
-	M17	fragment-like	[3.158]	2.93	267.11	283.7	2	24	45809595	CC(C)c1ccc(cc1)/C=C\2/c3ccccc3NC2=O
SM16	M18	fragment-like	[4.714, 9.847]	3.31	269.32	385	3	20	1284691	c1cc(c(c(c1)Cl)C(=O)Nc2ccncc2)Cl
SM17	M19	fragment-like	[4.902]	3.34	426.44	170	4	30	1444229	c1ccc(cc1)CSc2nnc(o2)c3ccncc3
SM18	D01	drug-like	[9.381, 10.773]	5.17	381.28	247.7	7	37	18897105	c1ccc2c(c1)c(=O)[nH]c(n2)CCC(=O)Nc3ncc(s3)Cc4ccc(c(c4)F)F
SM19	D02	drug-like	[9.167]	5.78	403.31	489.9	6	28	3365457	CCOc1ccc2c(c1)sc(n2)NC(=O)Cc3ccc(c(c3)Cl)Cl
-	D03	drug-like	[4.113]	4.72	401.48	324.5	6	35	10794751	CC(C)(C)c1cc(n(n1)c2ccccc2)NC(=O)Nc3cccc(c3Cl)Cl
-	D04	drug-like	[3.199]	5.24	380.25	636.9	5	34	3064762	c1ccc(cc1)C(=O)Nc2ccc(cc2)Oc3c4c5c(sc4ncn3)CCCC5
SM20	D05	drug-like	[8.05]	4.14	438.09	154	4	24	46568819	c1cc(cc(c1)OCc2ccc(cc2Cl)Cl)/C=C/3\C(=O)NC(=O)S3
SM21	D06	drug-like	[3.892]	3.37	396.95	222	4	28	1574612	c1cc(cc(c1)Br)Nc2c(cnc(n2)Nc3cccc(c3)Br)F
SM22	D07	drug-like	[3.511, 6.794]	2.94	420.46	239	0	29	536848	c1cc2c(cc(c(c2nc1)O)I)I
SM23	D08	drug-like	[6.336]	2.79	391.42	319	10	28	4375254	CCOC(=O)c1ccc(cc1)Nc2cc(nc(n2)Nc3ccc(cc3)C(=O)OCC)C
SM24	D09	drug-like	[4.829]	3.27	289.26	398	7	71	1415746	COc1ccc(cc1)c2c3c(ncnc3oc2c4ccc(cc4)OC)NCCO

Table SI 3. pK_a results of replicate UV-metric pKa measurements. "UV-metric pK_a" assay indicates spectrophotometric pK_a measurements done with Sirius T3 in ISA water. "UV-metric pKa with cosolvent" assay refers to pK_a determination by Yasuda-Shedlovsky extrapolation from p_sK_a measurements in various ratios of ISA methanol:water mixtures. Triplicate measurements were performed at 25 ± 0.5°C and in the presence of approximately 150 mM KCl to adjust ionic strength.

Molecule ID	pK_{a1}	pK_{a2}	pK_{a3}	Assay Type	Experiment ID	Experiment Report
SM01	9.54			UV-metric pK_a	17I-15024	SM01_17I-15024_M01_UV-metric pKa_report.pdf
SM01	9.53			UV-metric pK_a	17I-15025	SM01_17I-15025_M01_UV-metric pKa_report.pdf
SM01	9.53			UV-metric pK_a	17I-16001	SM01_17I-16001_M01_UV-metric pKa_report.pdf
SM02	5.04			UV-metric pK_a with cosolvent	17I-22022	SM02_17I-22022_M02_UV-metric psKa_report.pdf
SM02	5.04			UV-metric pK_a with cosolvent	17I-22023	SM02_17I-22023_M02_UV-metric psKa_report.pdf
SM02	5.02			UV-metric pK_a with cosolvent	17I-22024	SM02_17I-22024_M02_UV-metric psKa_report.pdf
SM03	7.01			UV-metric pK_a with cosolvent	17I-19004	SM03_17I-19004_M03_UV-metric psKa_report.pdf
SM03	7.01			UV-metric pK_a with cosolvent	17I-19005	SM03_17I-19005_M03_UV-metric psKa_report.pdf
SM03	7.03			UV-metric pK_a with cosolvent	17I-19006	SM03_17I-19006_M03_UV-metric psKa_report.pdf
SM04	6.03			UV-metric pK_a	17I-18018	SM04_17I-18018_M04_UV-metric pKa_report.pdf
SM04	6.02			UV-metric pK_a	17I-18019	SM04_17I-18019_M04_UV-metric pKa_report.pdf
SM04	6.02			UV-metric pK_a	17I-18020	SM04_17I-18020_M04_UV-metric pKa_report.pdf
SM05	4.57			UV-metric pK_a with cosolvent	17I-19007	SM05_17I-19007_M05_UV-metric psKa_report.pdf
SM05	4.6			UV-metric pK_a with cosolvent	17I-19008	SM05_17I-19008_M05_UV-metric psKa_report.pdf
SM05	4.6			UV-metric pK_a with cosolvent	17I-19009	SM05_17I-19009_M05_UV-metric psKa_report.pdf
SM06	2.96	11.76		UV-metric pK_a	17I-18021	SM06_17I-18021_M06_UV-metric pKa_report.pdf
SM06	3.11	11.74		UV-metric pK_a	17I-18022	SM06_17I-18022_M06_UV-metric pKa_report.pdf
SM06	3.02	11.71		UV-metric pK_a	17I-18023	SM06_17I-18023_M06_UV-metric pKa_report.pdf
SM07	6.07			UV-metric pK_a	17I-16002	SM07_17I-16002_M07_UV-metric pKa_report.pdf
SM07	6.07			UV-metric pK_a	17I-16004	SM07_17I-16004_M07_UV-metric pKa_report.pdf
SM07	6.09			UV-metric pK_a	17I-20001	SM07_17I-20001_M07_UV-metric pKa_report.pdf
SM08	4.23			UV-metric pK_a	17I-19001	SM08_17I-19001_M08_UV-metric pKa_report.pdf
SM08	4.2			UV-metric pK_a	17I-19002	SM08_17I-19002_M08_UV-metric pKa_report.pdf
SM08	4.22			UV-metric pK_a	17I-19003	SM08_17I-19003_M08_UV-metric pKa_report.pdf
SM09	5.37			UV-metric pK_a with cosolvent	17I-16014	SM09_17I-16014_M09_UV-metric psKa_report.pdf
SM09	5.35			UV-metric pK_a with cosolvent	17I-16015	SM09_17I-16015_M09_UV-metric psKa_report.pdf
SM09	5.4			UV-metric pK_a with cosolvent	17I-16016	SM09_17I-16016_M09_UV-metric psKa_report.pdf
SM10	9.01			UV-metric pK_a with cosolvent	17I-20020	SM10_17I-20020_M10_UV-metric psKa_report.pdf
SM10	9.02			UV-metric pK_a with cosolvent	17I-20021	SM10_17I-20021_M10_UV-metric psKa_report.pdf
SM10	9.02			UV-metric pK_a with cosolvent	17I-20022	SM10_17I-20022_M10_UV-metric psKa_report.pdf
SM11	3.89			UV-metric pK_a	17I-16005	SM11_17I-16005_M11_UV-metric pKa_report.pdf
SM11	3.89			UV-metric pK_a	17I-16006	SM11_17I-16006_M11_UV-metric pKa_report.pdf
SM11	3.89			UV-metric pK_a	17I-16007	SM11_17I-16007_M11_UV-metric pKa_report.pdf
SM12	5.28			UV-metric pK_a	17I-21002	SM12_17I-21002_M12_UV-metric pKa_report.pdf
SM12	5.28			UV-metric pK_a	17I-21003	SM12_17I-21003_M12_UV-metric pKa_report.pdf
SM12	5.27			UV-metric pK_a	17I-21004	SM12_17I-21004_M12_UV-metric pKa_report.pdf
SM13	5.79			UV-metric pK_a	17I-16011	SM13_17I-16011_M13_UV-metric pKa_report.pdf
SM13	5.76			UV-metric pK_a	17I-16012	SM13_17I-16012_M13_UV-metric pKa_report.pdf
SM13	5.76			UV-metric pK_a	17I-16013	SM13_17I-16013_M13_UV-metric pKa_report.pdf

Table SI 3. Continued.

Molecule ID	pK_{a1}	pK_{a2}	pK_{a3}	Assay Type	Experiment ID	Experiment Report
SM14	2.59	5.31		UV-metric pK_a	17I-29002	SM14_17I-29002_M15_UV-metric pKa_report.pdf
SM14	2.57	5.29		UV-metric pK_a	17I-29003	SM14_17I-29003_M15_UV-metric pKa_report.pdf
SM14	2.57	5.29		UV-metric pK_a	17I-29004	SM14_17I-29004_M15_UV-metric pKa_report.pdf
SM15	4.71	8.96		UV-metric pK_a	17K-10009	SM15_17K-10009_M16_UV-metric pKa_report.pdf
SM15	4.7	8.94		UV-metric pK_a	17K-10010	SM15_17K-10010_M16_UV-metric pKa_report.pdf
SM15	4.69	8.92		UV-metric pK_a	17K-10011	SM15_17K-10011_M16_UV-metric pKa_report.pdf
SM16	5.37	10.64		UV-metric pK_a	17J-03025	SM16_17J-03025_M18_UV-metric pKa_report.pdf
SM16	5.37	10.65		UV-metric pK_a	17J-03026	SM16_17J-03026_M18_UV-metric pKa_report.pdf
SM16	5.38	10.65		UV-metric pK_a	17J-03027	SM16_17J-03027_M18_UV-metric pKa_report.pdf
SM17	3.15			UV-metric pK_a	17J-02024	SM17_17J-02024_M19_UV-metric pKa_report.pdf
SM17	3.16			UV-metric pK_a	17J-02025	SM17_17J-02025_M19_UV-metric pKa_report.pdf
SM17	3.17			UV-metric pK_a	17J-02026	SM17_17J-02026_M19_UV-metric pKa_report.pdf
SM18	2.1	9.51	10.93	UV-metric pK_a with cosolvent	17I-28001	SM18_17I-28001_D01_UV-metric psKa_report.pdf
SM18	2.15	9.59	11.03	UV-metric pK_a with cosolvent	17I-28002	SM18_17I-28002_D01_UV-metric psKa_report.pdf
SM18	2.19	9.63	11.11	UV-metric pK_a with cosolvent	17I-28003	SM18_17I-28003_D01_UV-metric psKa_report.pdf
SM19	9.59			UV-metric pK_a with cosolvent	17I-28004	SM19_17I-28004_D02_UV-metric psKa_report.pdf
SM19	9.58			UV-metric pK_a with cosolvent	17I-28005	SM19_17I-28005_D02_UV-metric psKa_report.pdf
SM19	9.52			UV-metric pK_a with cosolvent	17I-28006	SM19_17I-28006_D02_UV-metric psKa_report.pdf
SM20	5.72			UV-metric pK_a with cosolvent	17J-12002	SM20_17J-12002_D05_UV-metric psKa_report.pdf
SM20	5.75			UV-metric pK_a with cosolvent	17J-12003	SM20_17J-12003_D05_UV-metric psKa_report.pdf
SM20	5.64			UV-metric pK_a with cosolvent	17K-16013	SM20_17K-16013_D05_UV-metric psKa_report.pdf
SM21	4.11			UV-metric pK_a with cosolvent	17J-06002	SM21_17J-06002_D06_UV-metric psKa_report.pdf
SM21	4.09			UV-metric pK_a with cosolvent	17J-06003	SM21_17J-06003_D06_UV-metric psKa_report.pdf
SM21	4.09			UV-metric pK_a with cosolvent	17J-06004	SM21_17J-06004_D06_UV-metric psKa_report.pdf
SM22	2.45	7.42		UV-metric pK_a with cosolvent	17J-07003	SM22_17J-07003_D07_UV-metric psKa_report.pdf
SM22	2.37	7.45		UV-metric pK_a with cosolvent	17J-07004	SM22_17J-07004_D07_UV-metric psKa_report.pdf
SM22	2.37	7.41		UV-metric pK_a with cosolvent	17J-07005	SM22_17J-07005_D07_UV-metric psKa_report.pdf
SM23	5.45			UV-metric pK_a with cosolvent	17J-07006	SM23_17J-07006_D08_UV-metric psKa_report.pdf
SM23	5.44			UV-metric pK_a with cosolvent	17J-07007	SM23_17J-07007_D08_UV-metric psKa_report.pdf
SM23	5.45			UV-metric pK_a with cosolvent	17J-07008	SM23_17J-07008_D08_UV-metric psKa_report.pdf
SM24	2.61			UV-metric pK_a with cosolvent	17J-06007	SM24_17J-06007_D09_UV-metric psKa_report.pdf
SM24	2.58			UV-metric pK_a with cosolvent	17J-06008	SM24_17J-06008_D09_UV-metric psKa_report.pdf
SM24	2.6			UV-metric pK_a with cosolvent	17J-06009	SM24_17J-06009_D09_UV-metric psKa_report.pdf

Table SI 4. UV-metric pK_a measurements with and without cosolvent for 12 pK_a challenge compounds with higher aqueous solubility and pyridoxine HCl. pK_a values were measured with both methods to test if cosolvent method introduces bias.

Molecule ID	pK_{a1}	pK_{a2}	pK_{a3}	Assay Type	Experiment ID	Experiment Report
SM01	9.54			UV-metric pK_a	17I-15024	SM01_17I-15024_M01_UV-metric pKa_report.pdf
SM01	9.53			UV-metric pK_a	17I-15025	SM01_17I-15025_M01_UV-metric pKa_report.pdf
SM01	9.53			UV-metric pK_a	17I-16001	SM01_17I-16001_M01_UV-metric pKa_report.pdf
SM01*	9.71			UV-metric pK_a with cosolvent	17J-06011	SM01_17J-06011_M01_UV-metric psKa_report.pdf
SM04	6.03			UV-metric pK_a	17I-18018	SM04_17I-18018_M04_UV-metric pKa_report.pdf
SM04	6.02			UV-metric pK_a	17I-18019	SM04_17I-18019_M04_UV-metric pKa_report.pdf
SM04	6.02			UV-metric pK_a	17I-18020	SM04_17I-18020_M04_UV-metric pKa_report.pdf
SM04*	5.97			UV-metric pK_a with cosolvent	17K-09020	SM04_17K-09020_M04_UV-metric psKa_report.pdf
SM06	2.96	11.76		UV-metric pK_a	17I-18021	SM06_17I-18021_M06_UV-metric pKa_report.pdf
SM06	3.11	11.74		UV-metric pK_a	17I-18022	SM06_17I-18022_M06_UV-metric pKa_report.pdf
SM06	3.02	11.71		UV-metric pK_a	17I-18023	SM06_17I-18023_M06_UV-metric pKa_report.pdf
SM06*	3.46	>12		UV-metric pK_a with cosolvent	17K-09021	SM06_17K-09021_M06_UV-metric psKa_report.pdf
SM06*	3.4	>12		UV-metric pK_a with cosolvent	17K-10008	SM06_17K-10008_M06_UV-metric psKa_report.pdf
SM06*	3.45	>12		UV-metric pK_a with cosolvent	17K-16011	SM06_17K-16011_M06_UV-metric psKa_report.pdf
SM07	6.07			UV-metric pK_a	17I-16002	SM07_17I-16002_M07_UV-metric pKa_report.pdf
SM07	6.07			UV-metric pK_a	17I-16004	SM07_17I-16004_M07_UV-metric pKa_report.pdf
SM07	6.09			UV-metric pK_a	17I-20001	SM07_17I-20001_M07_UV-metric pKa_report.pdf
SM07*	5.96			UV-metric pK_a with cosolvent	17J-12006	SM07_17J-12006_M07_UV-metric psKa_report.pdf
SM08	4.23			UV-metric pK_a	17I-19001	SM08_17I-19001_M08_UV-metric pKa_report.pdf
SM08	4.2			UV-metric pK_a	17I-19002	SM08_17I-19002_M08_UV-metric pKa_report.pdf
SM08	4.22			UV-metric pK_a	17I-19003	SM08_17I-19003_M08_UV-metric pKa_report.pdf
SM08*	4.47			UV-metric pK_a with cosolvent	17J-12007	SM08_17J-12007_M08_UV-metric psKa_report.pdf
SM11	3.89			UV-metric pK_a	17I-16005	SM11_17I-16005_M11_UV-metric pKa_report.pdf
SM11	3.89			UV-metric pK_a	17I-16006	SM11_17I-16006_M11_UV-metric pKa_report.pdf
SM11	3.89			UV-metric pK_a	17I-16007	SM11_17I-16007_M11_UV-metric pKa_report.pdf
SM11*	3.74			UV-metric pK_a with cosolvent	17J-04003	SM11_17J-04003_M11_UV-metric psKa_report.pdf
SM12	5.28			UV-metric pK_a	17I-21002	SM12_17I-21002_M12_UV-metric pKa_report.pdf
SM12	5.28			UV-metric pK_a	17I-21003	SM12_17I-21003_M12_UV-metric pKa_report.pdf
SM12	5.27			UV-metric pK_a	17I-21004	SM12_17I-21004_M12_UV-metric pKa_report.pdf
SM12*	5.16			UV-metric pK_a with cosolvent	17J-11014	SM12_17J-11014_M12_UV-metric psKa_report.pdf
SM13	5.79			UV-metric pK_a	17I-16011	SM13_17I-16011_M13_UV-metric pKa_report.pdf
SM13	5.76			UV-metric pK_a	17I-16012	SM13_17I-16012_M13_UV-metric pKa_report.pdf
SM13	5.76			UV-metric pK_a	17I-16013	SM13_17I-16013_M13_UV-metric pKa_report.pdf
SM13*	5.68			UV-metric pK_a with cosolvent	17J-04005	SM13_17J-04005_M13_UV-metric psKa_report.pdf
SM14	2.59	5.31		UV-metric pK_a	17I-29002	SM14_17I-29002_M15_UV-metric pKa_report.pdf
SM14	2.57	5.29		UV-metric pK_a	17I-29003	SM14_17I-29003_M15_UV-metric pKa_report.pdf
SM14	2.57	5.29		UV-metric pK_a	17I-29004	SM14_17I-29004_M15_UV-metric pKa_report.pdf
SM14*	2.56	5.29		UV-metric pK_a with cosolvent	17J-12008	SM14_17J-12008_M15_UV-metric psKa_report.pdf

* These UV-metric pKa measurement with cosolvent were not used in the calculation of pK_a mean and SEM in the experimental data reported fo SAMPL6 pK_a challenge because replicate water based experiments also existed for these molecules.

Table SI 4. Continued.

Molecule ID	pK_{a1}	pK_{a2}	pK_{a3}	Assay Type	Experiment ID	Experiment Report
SM15	4.71	8.96		UV-metric pK_a	17K-10009	SM15_17K-10009_M16_UV-metric pKa_report.pdf
SM15	4.7	8.94		UV-metric pK_a	17K-10010	SM15_17K-10010_M16_UV-metric pKa_report.pdf
SM15	4.69	8.92		UV-metric pK_a	17K-10011	SM15_17K-10011_M16_UV-metric pKa_report.pdf
SM15*	4.67	9.01		UV-metric pK_a with cosolvent	17J-12009	SM15_17J-12009_M16_UV-metric psKa_report.pdf
SM16	5.37	10.64		UV-metric pK_a	17J-03025	SM16_17J-03025_M18_UV-metric pKa_report.pdf
SM16	5.37	10.65		UV-metric pK_a	17J-03026	SM16_17J-03026_M18_UV-metric pKa_report.pdf
SM16	5.38	10.65		UV-metric pK_a	17J-03027	SM16_17J-03027_M18_UV-metric pKa_report.pdf
SM16*	5.35	11.01		UV-metric pK_a with cosolvent	17J-12010	SM16_17J-12010_M18_UV-metric psKa_report.pdf
SM17	3.15			UV-metric pK_a	17J-02024	SM17_17J-02024_M19_UV-metric pKa_report.pdf
SM17	3.16			UV-metric pK_a	17J-02025	SM17_17J-02025_M19_UV-metric pKa_report.pdf
SM17	3.17			UV-metric pK_a	17J-02026	SM17_17J-02026_M19_UV-metric pKa_report.pdf
SM17*	3.2			UV-metric pK_a with cosolvent	17J-12011	SM17_17J-12011_M19_UV-metric psKa_report.pdf
Pyridoxine HCl	4.81	8.85		UV-metric pK_a	18E-22003	18E-22003_Pyridoxine HCl_UV-metric pKa_0417936-0002.pdf
Pyridoxine HCl	4.83	8.87		UV-metric pK_a	18E-22004	18E-22004_Pyridoxine HCl_UV-metric pKa_0417936-0002.pdf
Pyridoxine HCl	4.84	8.86		UV-metric pK_a	18E-22010	18E-22010_Pyridoxine HCl_UV-metric pKa_0417936-0002.pdf
Pyridoxine HCl	4.85	8.79		UV-metric pK_a with cosolvent	18E-22011	18E-22011_Pyridoxine HCl_UV-metric psKa_0417936-0002.pdf
Pyridoxine HCl	4.85	8.79		UV-metric pK_a with cosolvent	18E-22012	18E-22012_Pyridoxine HCl_UV-metric psKa_0417936-0002.pdf
Pyridoxine HCl	4.86	8.77		UV-metric pK_a with cosolvent	18E-22013	18E-22013_Pyridoxine HCl_UV-metric psKa_0417936-0002.pdf

* These UV-metric pKa measurement with cosolvent were not used in the calculation of pKa mean and SEM in the experimental data reported for SAMPL6 pKa challenge because replicate water based experiments also existed for these molecules.

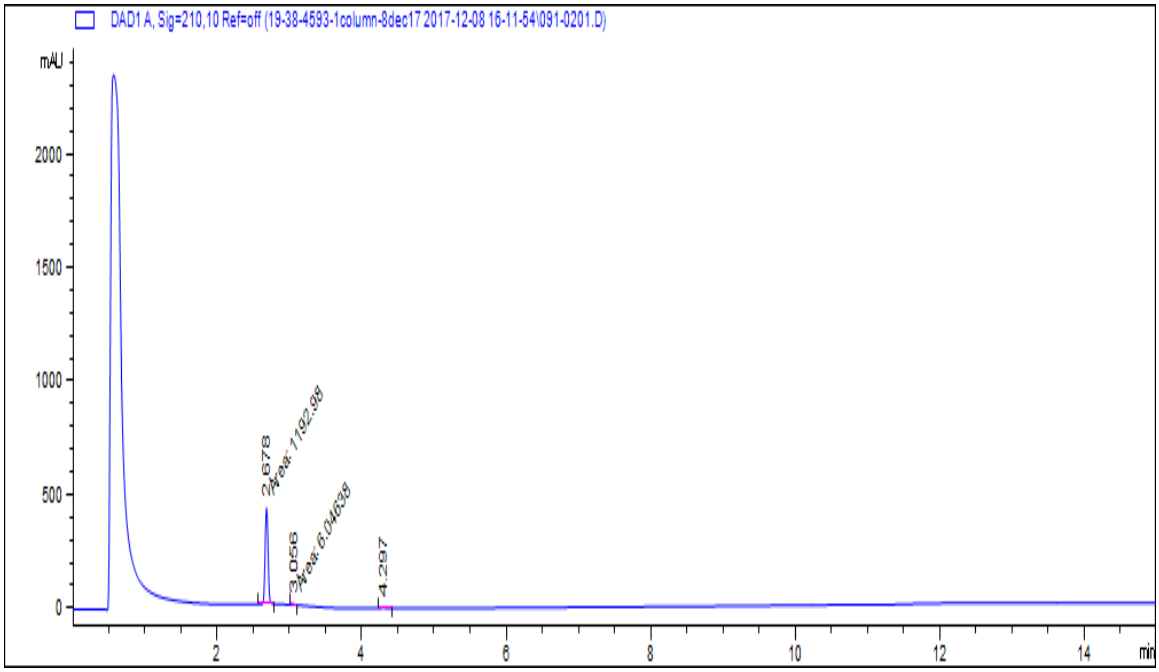
Table SI 5. pK_a mean and SEM results of with (cosolvent) and without cosolvent (water) replicate experiment. pKa values without SEM reported were measured with 1 replicate. pK_a values reported with SEM were measured in triplicates.

Molecule ID	pK_a ID	pK_a mean water	pK_a SEM water	pK_a mean cosolvent	pK_a SEM cosolvent
SM01	SM01_pKa1	9.53	0.01	9.71	
SM04	SM04_pKa1	6.02	0.01	5.97	
SM06	SM06_pKa1	3.03	0.04	3.44	0.02
SM07	SM07_pKa1	6.08	0.01	5.96	
SM08	SM08_pKa1	4.22	0.01	4.47	
SM11	SM11_pKa1	3.89	0.01	3.74	
SM12	SM12_pKa1	5.28	0.01	5.16	
SM13	SM13_pKa1	5.77	0.01	5.68	
SM14	SM14_pKa1	2.58	0.01	2.56	
SM14	SM14_pKa2	5.3	0.01	5.29	
SM15	SM15_pKa1	4.7	0.01	4.67	
SM15	SM15_pKa2	8.94	0.01	9.01	
SM16	SM16_pKa1	5.37	0.01	5.35	
SM16	SM16_pKa2	10.65	0.01	11.01	
SM17	SM17_pKa1	3.16	0.01	3.2	
Pyridoxine HCl	Pyridoxine HCl_pKa1	4.83	0.01	4.85	0.01
Pyridoxine HCl	Pyridoxine HCl_pKa2	8.86	0.01	8.78	0.01

Table SI 6. Summary of LC-MS purity results of SAMPL6 pK_a challenge compounds. LC-MS purity was calculated as percentage of area under the curve relative to all the peaks observed.

SAMPL6 molecule ID	Supplier reported purity (%)	LC-MS purity (Area%)	Supplier reported molecular weight (g/mol)	eMolecules reported molecular weight (g/mol)	LC/MS molecular weight (g/mol)	eMolecules ID
SM01	95	98.516	217.23	217.221	217.221	6679830
SM02		97.62		289.255	289.255	1327907
SM03		90.798		301.387	301.387	1228629
SM04		99.884		269.729	269.729	30719859
SM05		98.749		304.771	304.771	18908671
SM06		98.913		328.163	328.163	18893169
SM07	95	99.384	235.284	235.284	235.284	1327878
SM08	95	99.242	293.317	293.317	293.317	1367649
SM09	95	98.969	287.744	287.744	287.744	1865544
SM10	95	96.98	311.357	311.358	311.358	23354217
SM11	90	98.022		211.223	211.233	719540
SM12		98.134		292.163	292.163	1859493
SM13		97.512		295.336	295.336	5828805
SM14		96.484		209.247	209.247	31653344
SM15		98.698		210.231	210.231	37095168
SM16		97.733	267.114	267.111	267.111	1284691
SM17		96.994		269.322	269.322	1444229
SM18		92.601		426.439	426.439	18897105
SM19		94.464		381.276	381.276	3365457
SM20		97.176	380.25	380.245	380.245	46568819
SM21		96.791	438.1	438.092	438.092	1574612
SM22		86.975	396.95	396.951	396.951	536848
SM23		96.011	420.47	420.461	420.461	4375254
SM24		99.331	391.43	391.42	391.42	1415746

A



#	Retention time (minutes)	Area	Area%
1	2.7	1193	98.5
2	3.1	6	0.5
3	4.3	11.9	1.0

B

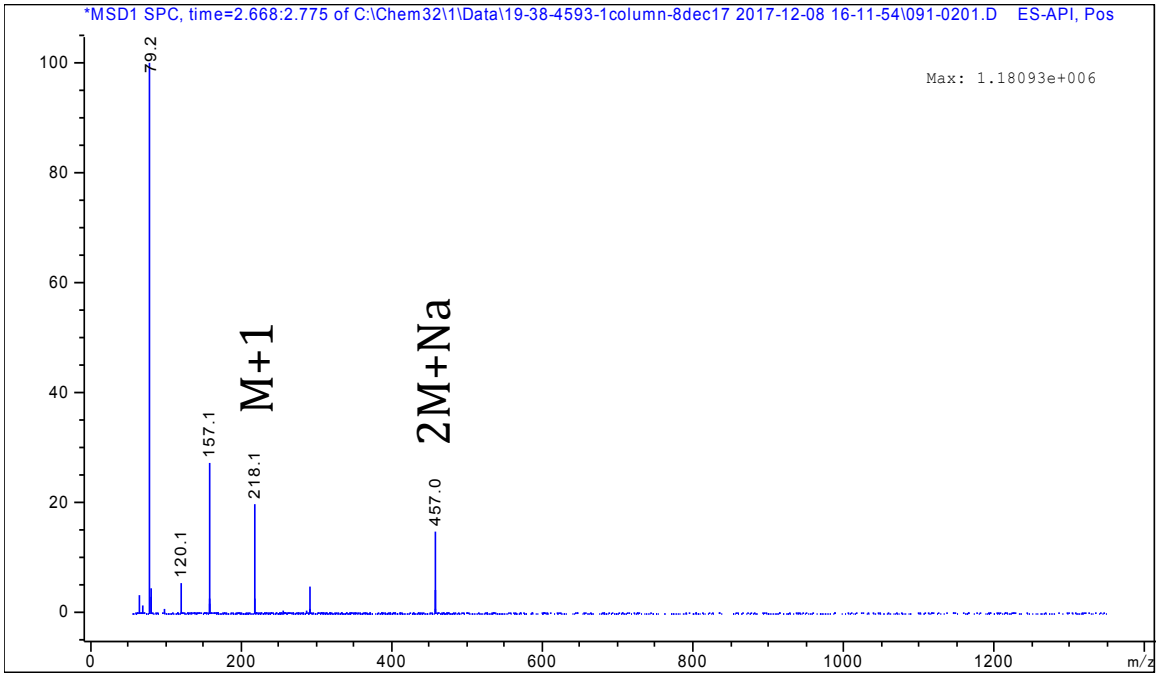
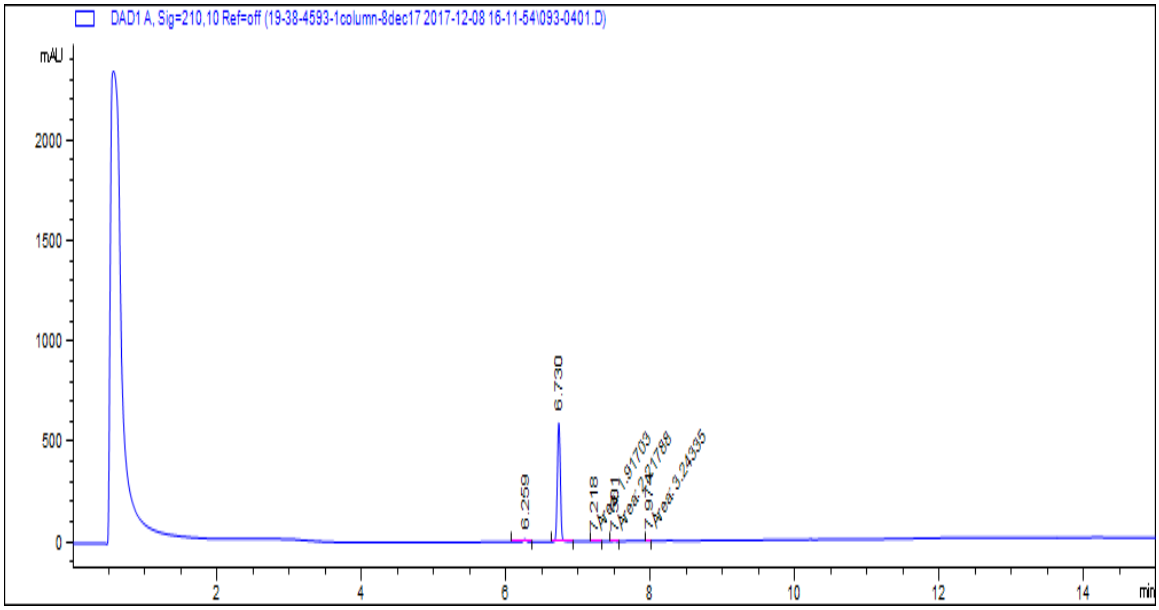


Figure SI 1. LC-MS chromatogram and mass spectrum for SM01. Molecular weight is 217.221 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B)Mass spectrum

A



#	Retention time (minutes)	Area	Area%
1	6.3	34.9	2.0
2	6.7	1733.8	97.6
3	7.2	1.9	0.1
4	7.5	2.2	0.1
5	8.0	3.2	0.2

B

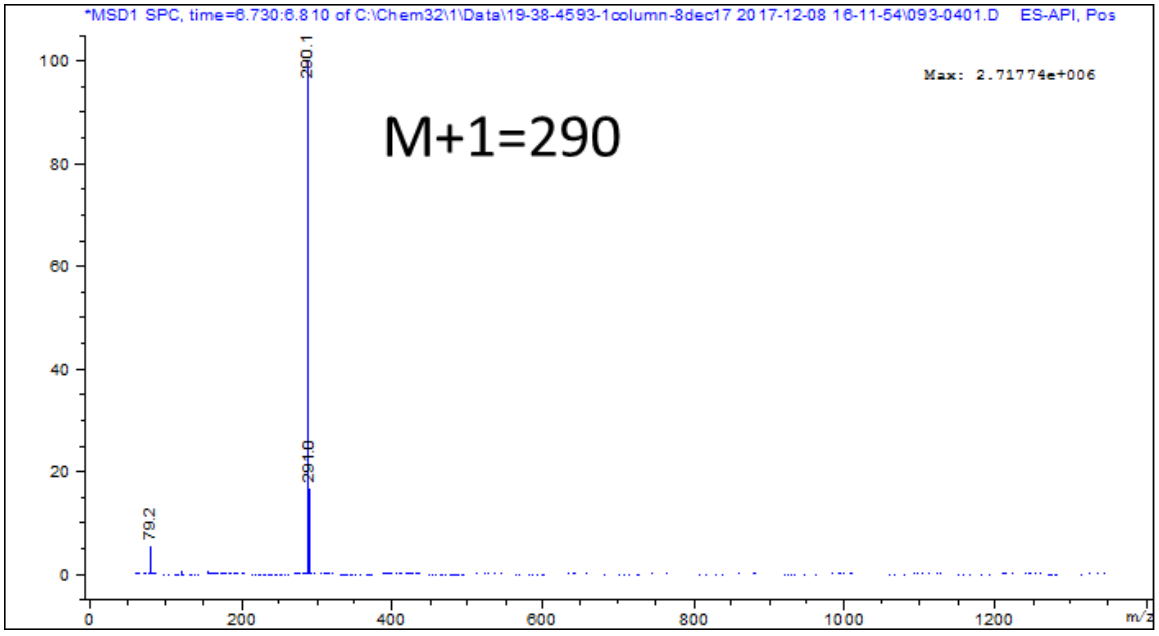
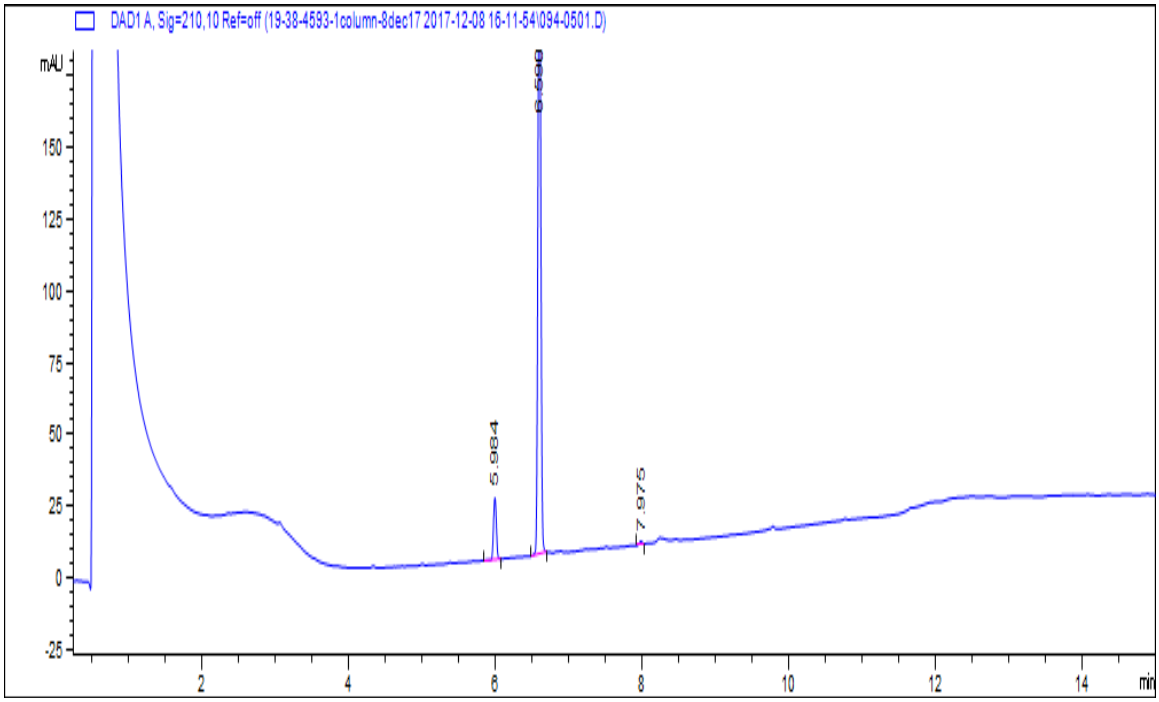


Figure SI 2. LC-MS chromatogram and mass spectrum for SM02. Molecular weight is 289.255 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

A



#	Retention time (minutes)	Area	Area%
1	6.0	58.2	8.7
2	6.6	606.7	90.8
3	8.0	3.3	0.5

B

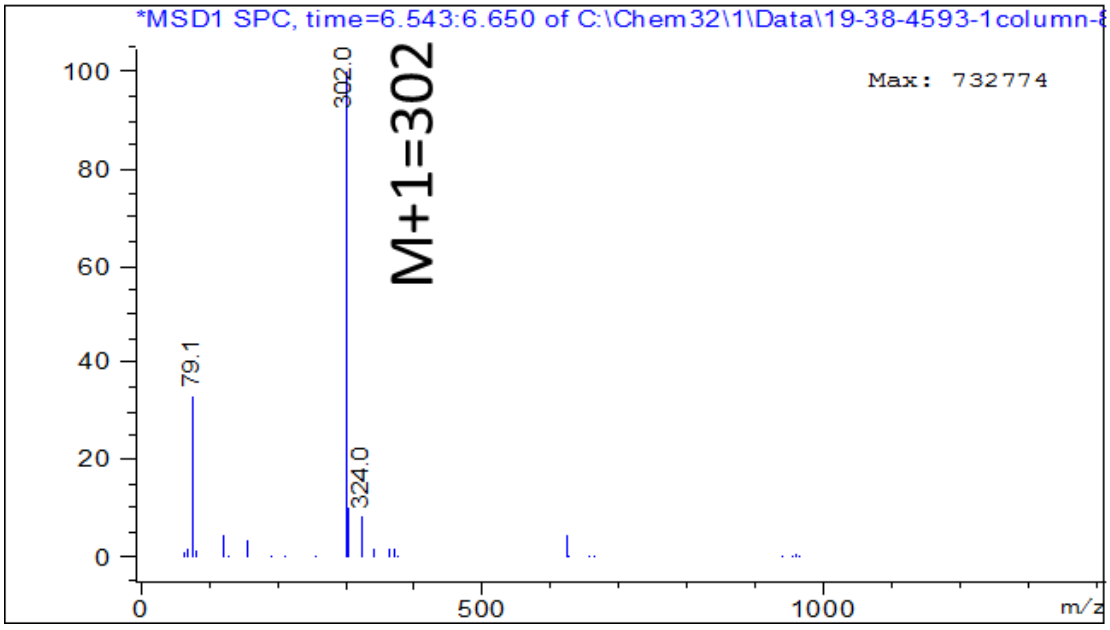
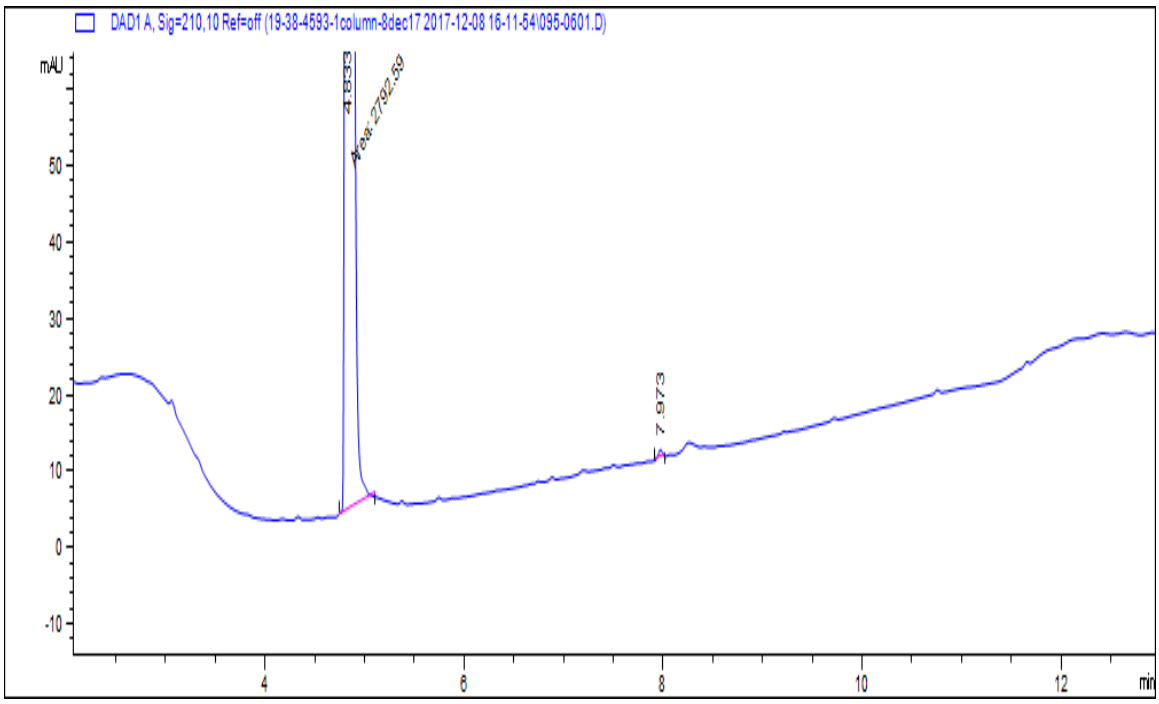


Figure SI 3. LC-MS chromatogram and mass spectrum for SM03. Molecular weight is 301.387 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

A



#	Retention time (minutes)	Area	Area%
1	4.8	2792.6	99.9
2	8.0	3.2	0.1

B

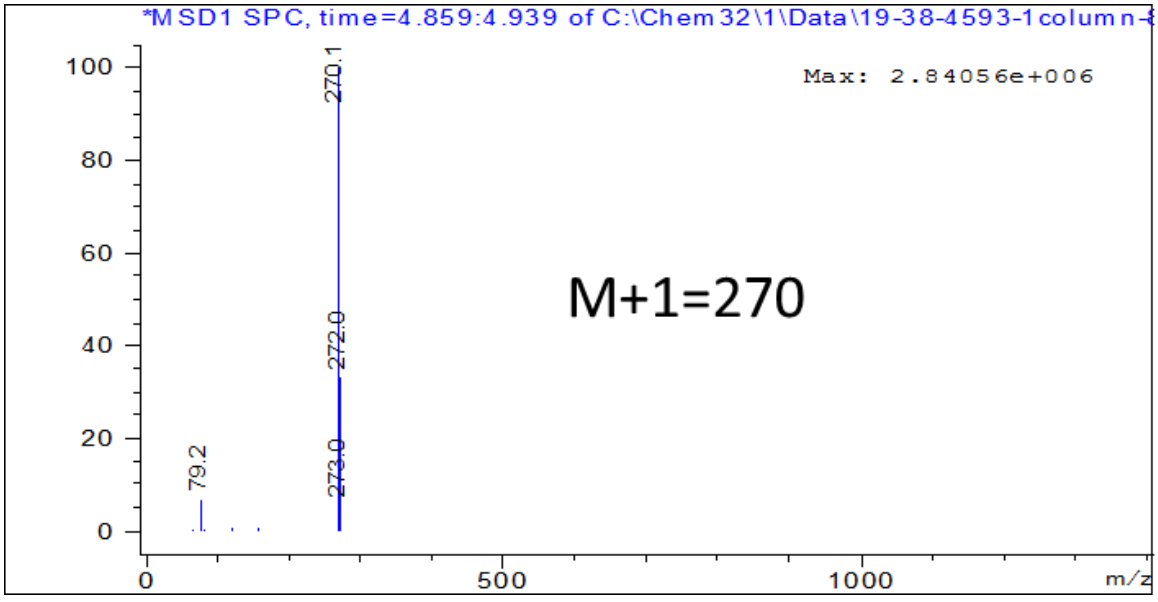
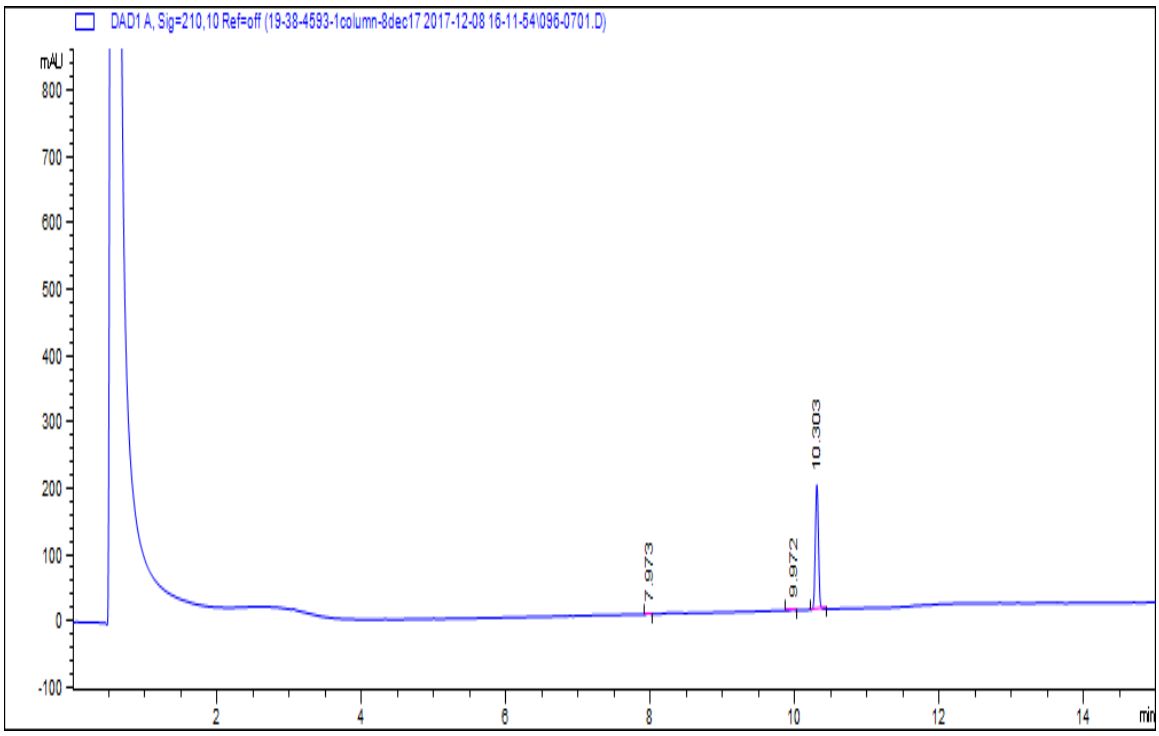


Figure SI 4. LC-MS chromatogram and mass spectrum for SM04. Molecular weight is 269.729 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

A



#	Retention time (minutes)	Area	Area%
1	8.0	3.3	0.6
2	10.0	4	0.7
3	10.3	569.7	98.7

B

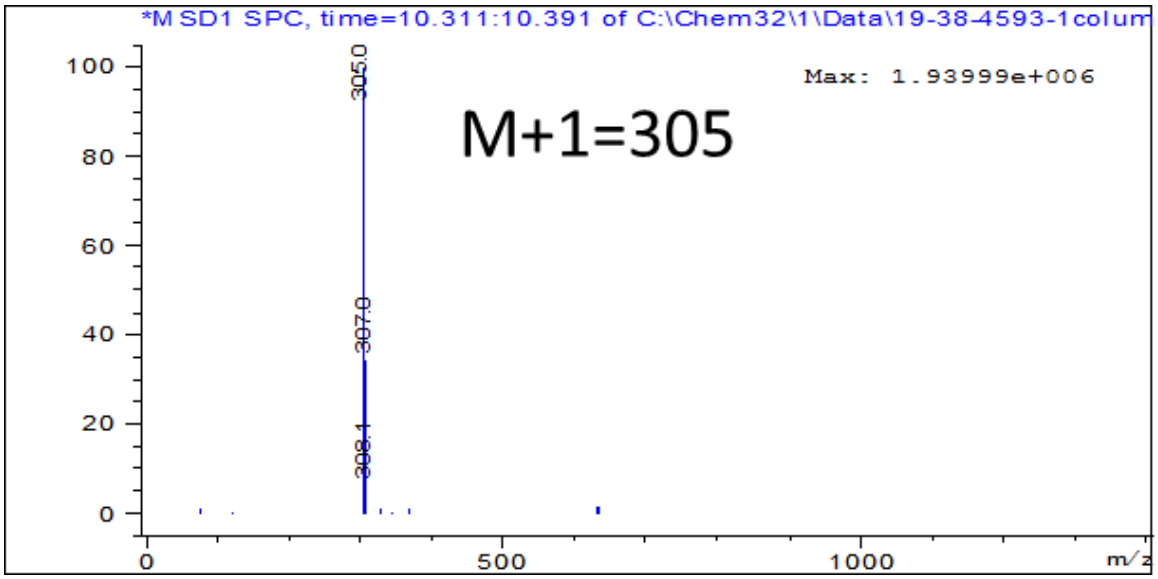
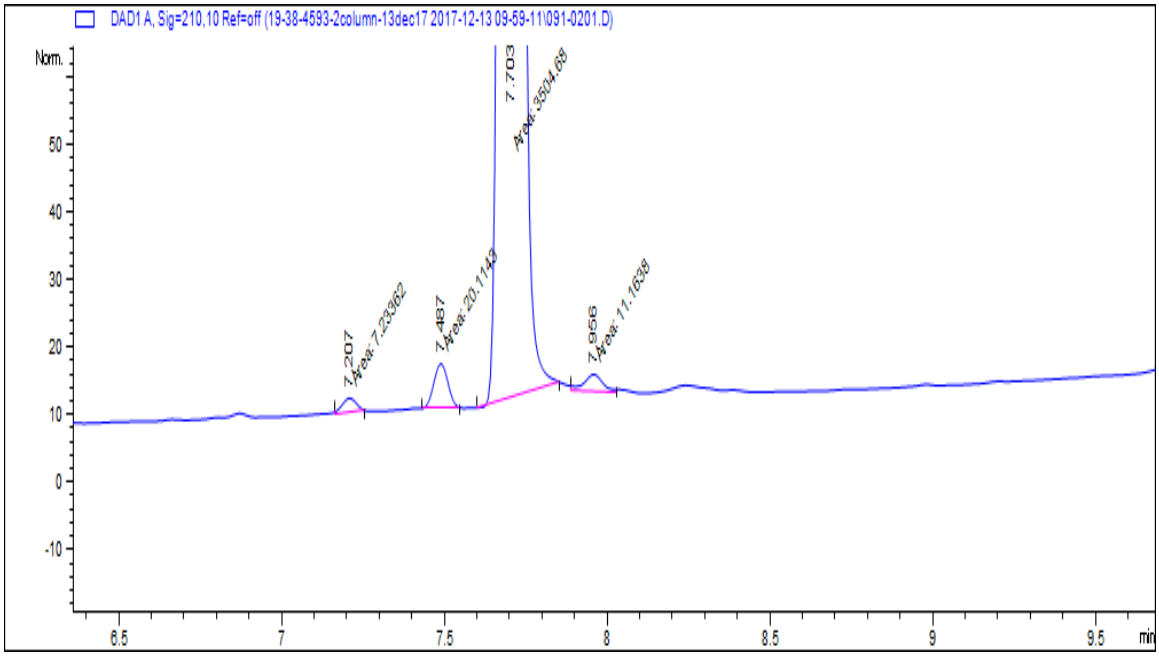


Figure SI 5. LC-MS chromatogram and mass spectrum for SM05. Molecular weight is 304.771 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

A



#	Retention time (minutes)	Area	Area%
1	7.2	7.2	0.2
2	7.5	20.1	0.6
3	7.7	3504.7	98.9
4	8.0	11.2	0.3

B

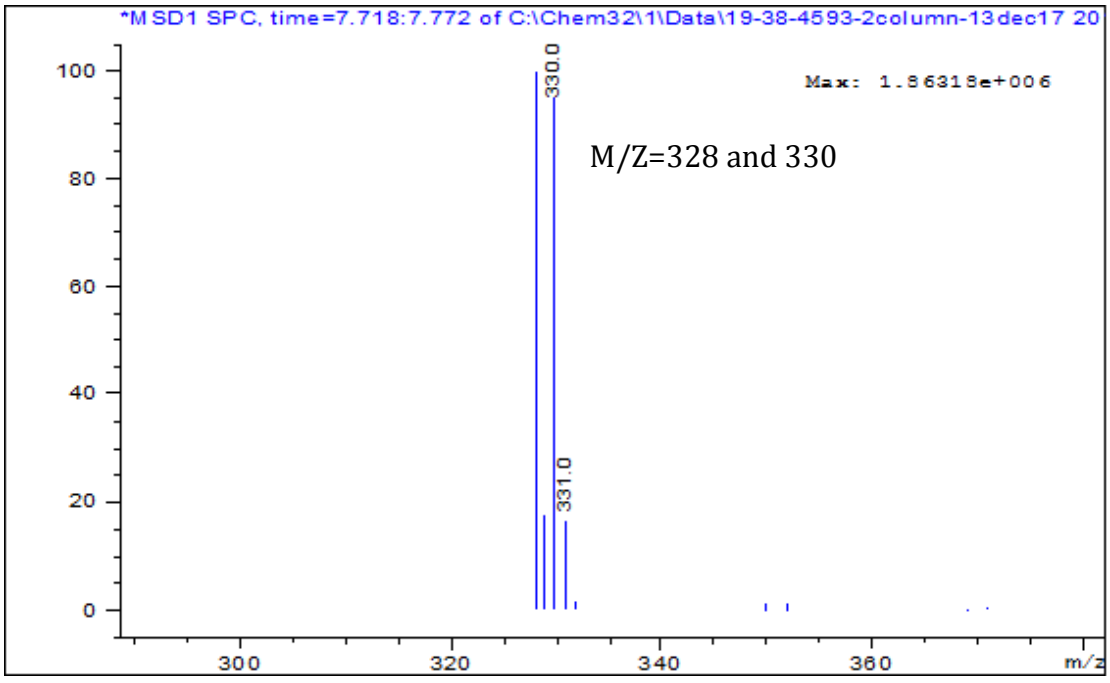
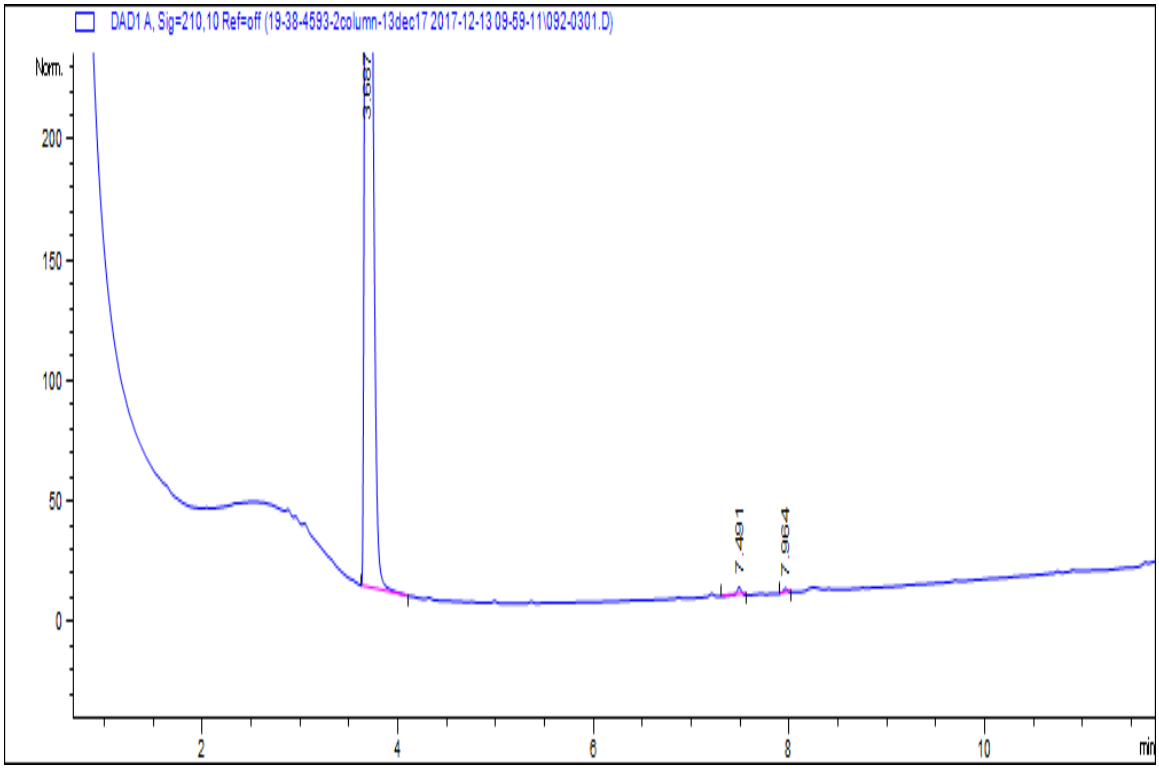


Figure SI 6. LC-MS chromatogram and mass spectrum for SM06. Molecular weight is 328.163 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

A



#	Retention time (minutes)	Area	Area%
1	3.7	2822.4	99.4
2	7.5	11.3	0.4
3	8.0	6.2	0.2

B

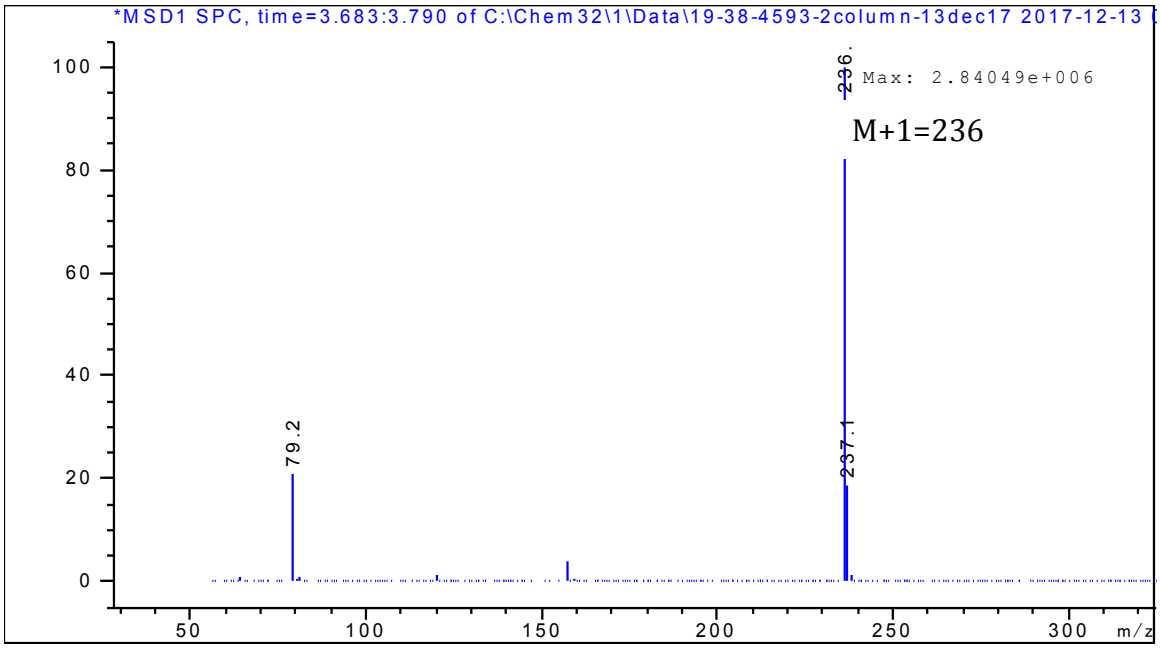
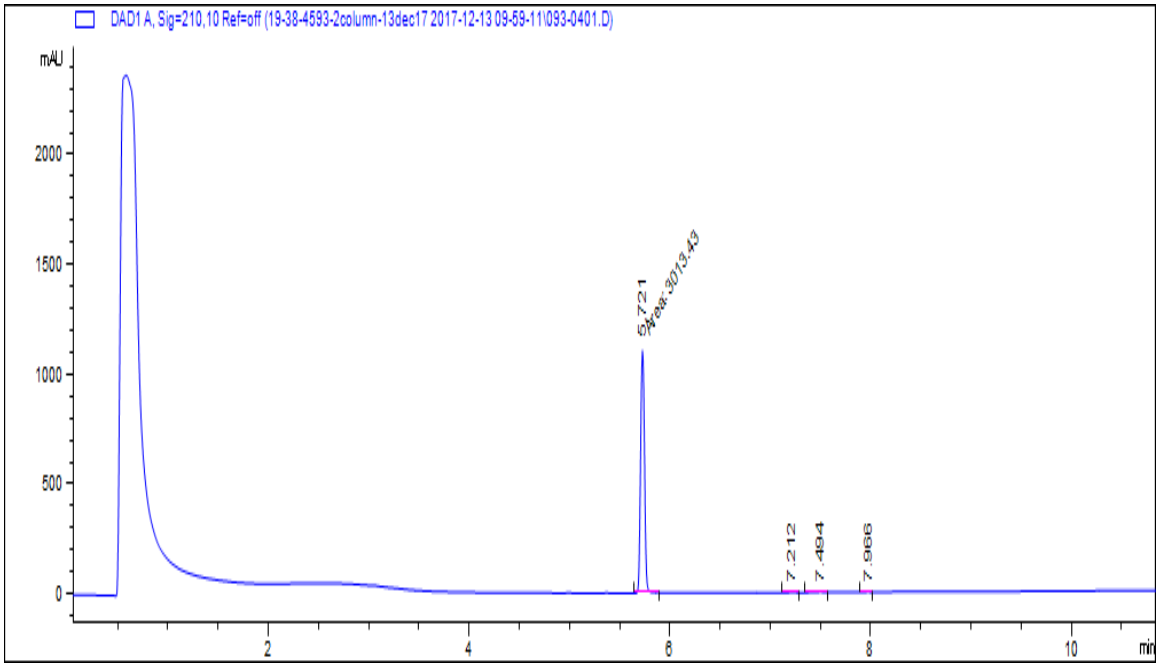


Figure SI 7. LC-MS chromatogram and mass spectrum for SM07. Molecular weight is 235.284 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

A



#	Retention time (minutes)	Area	Area%
1	5.7	3013.4	99.2
2	7.2	5	0.2
3	7.5	11.1	0.4
4	8.0	6.9	0.2

B

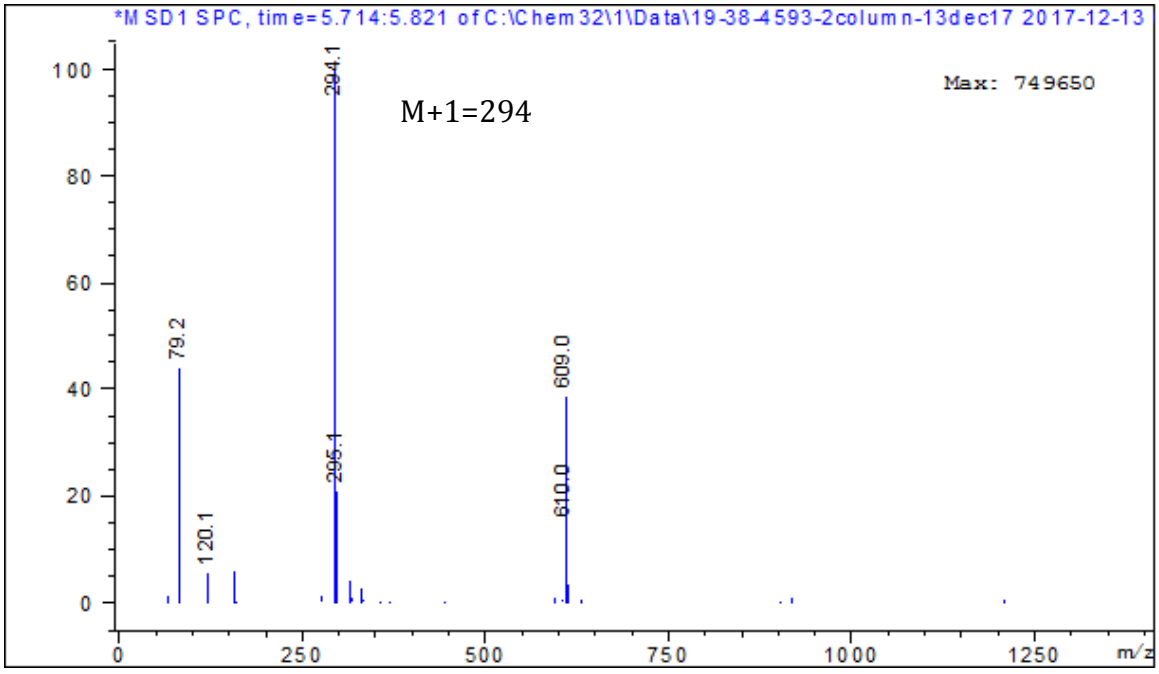
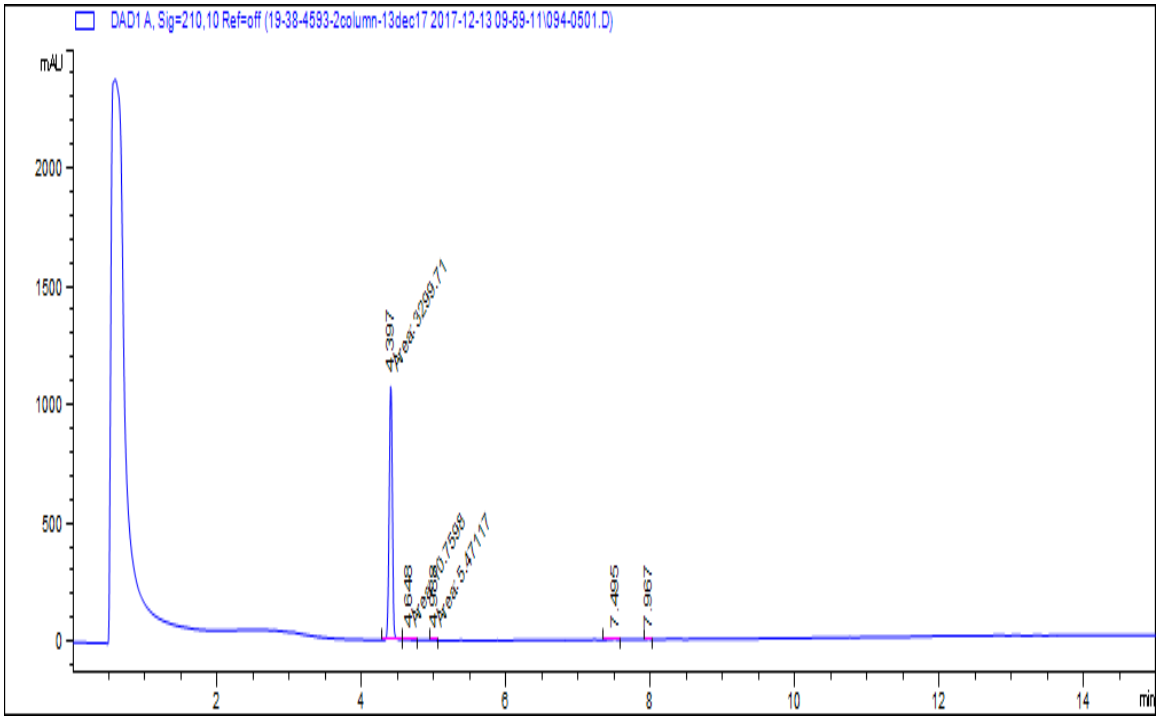


Figure SI 8. LC-MS chromatogram and mass spectrum for SM08. Molecular weight estimated from mass spectrometry is 293.317 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

A



#	Retention time (minutes)	Area	Area%
1	4.4	3299.7	99.0
2	4.6	10.8	0.3
3	5.0	5.5	0.2
4	7.5	12.6	0.4
5	8.0	5.7	0.2

B

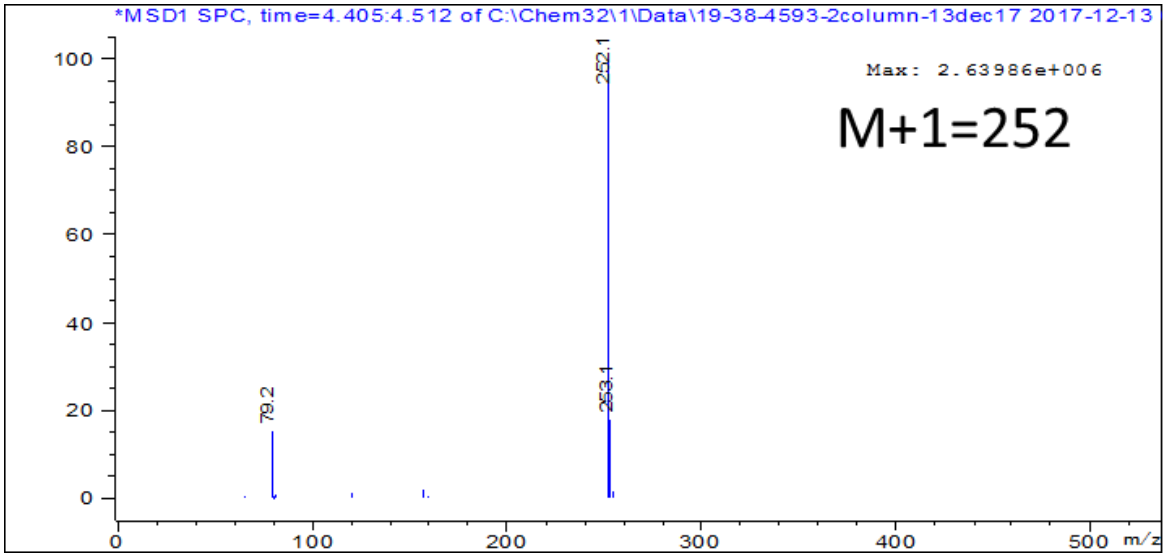
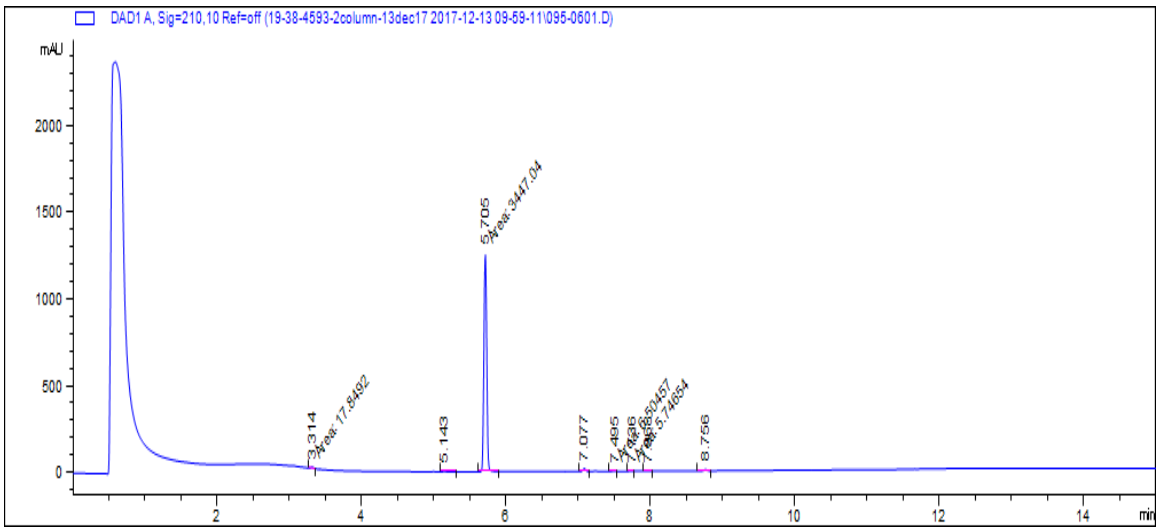


Figure SI 9. LC-MS chromatogram and mass spectrum for SM09. Molecular weight estimated from mass spectrometry is 287.744 g/mol (m/z = 251.244). (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

A



#	Retention time (minutes)	Area	Area%
2	5.1	5.2	0.1
3	5.7	3447	97.0
4	7.1	43.7	1.2
5	7.5	6.5	0.2
6	7.7	5.7	0.2
7	8.0	6.8	0.2
8	8.8	21.5	0.6

B

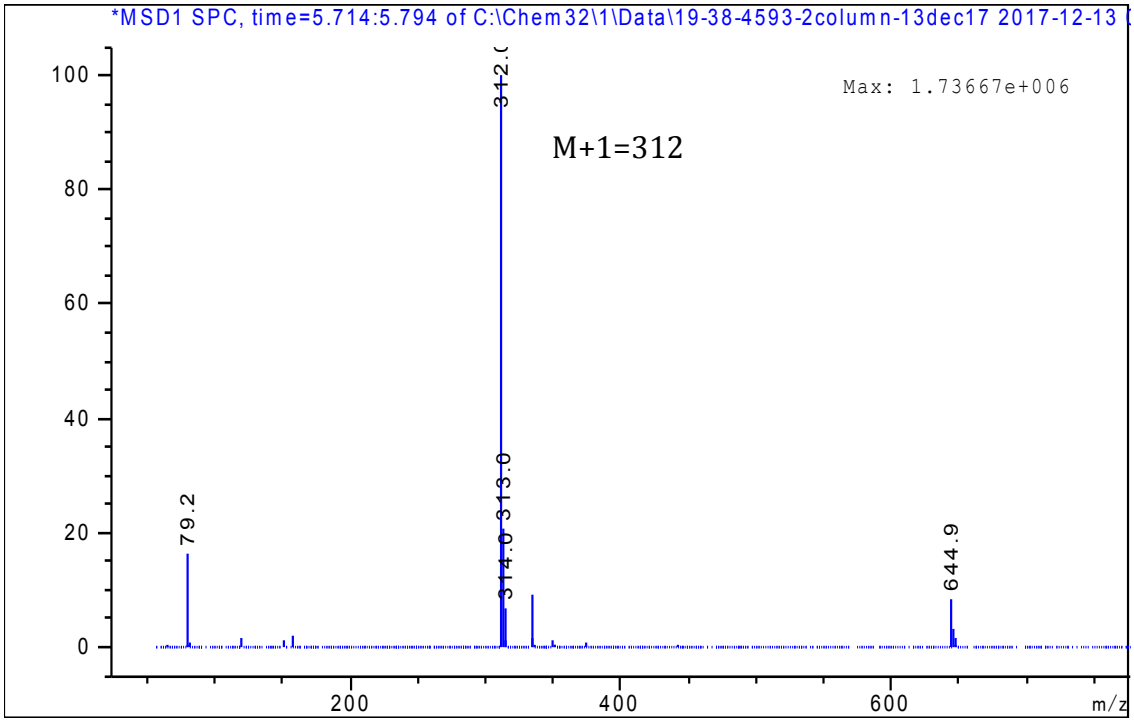
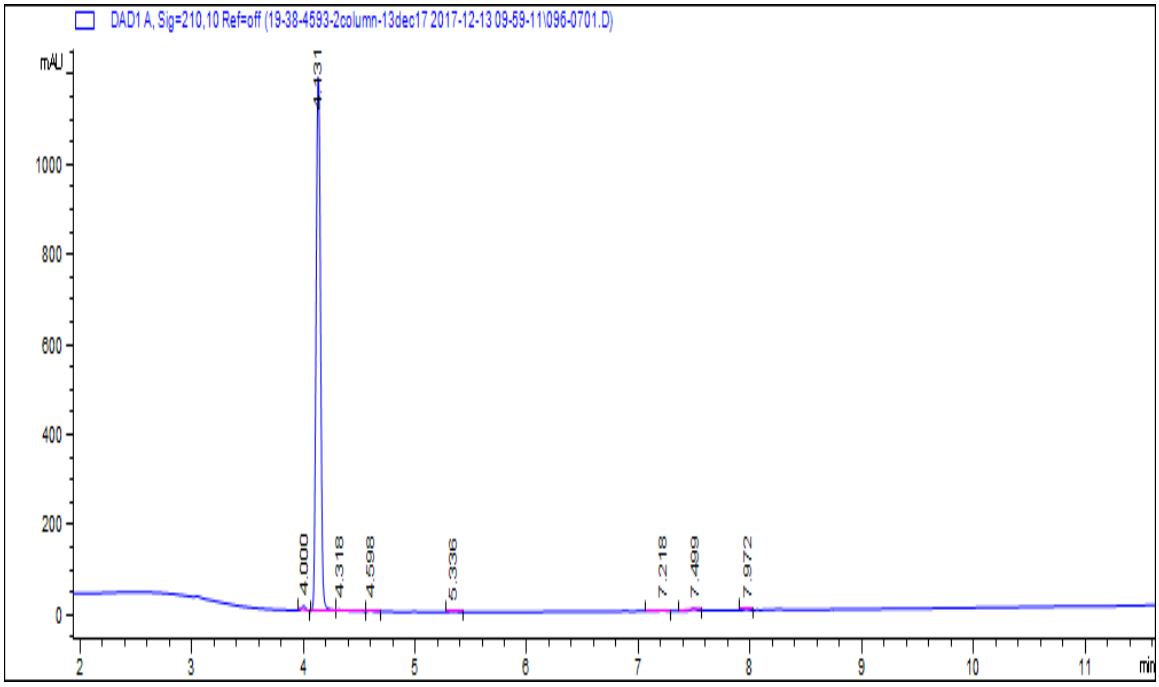


Figure SI 10. LC-MS chromatogram and mass spectrum for SM10. Molecular weight is 311.358 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

A



#	Retention time (minutes)	Area	Area%
1	4	25.1	0.8
2	4.1	3281.8	98.0
3	4.6	5.6	0.2
4	5.3	6.5	0.2
5	7.2	7.1	0.2
6	7.5	15.2	0.5
7	8.0	6.8	0.2

B

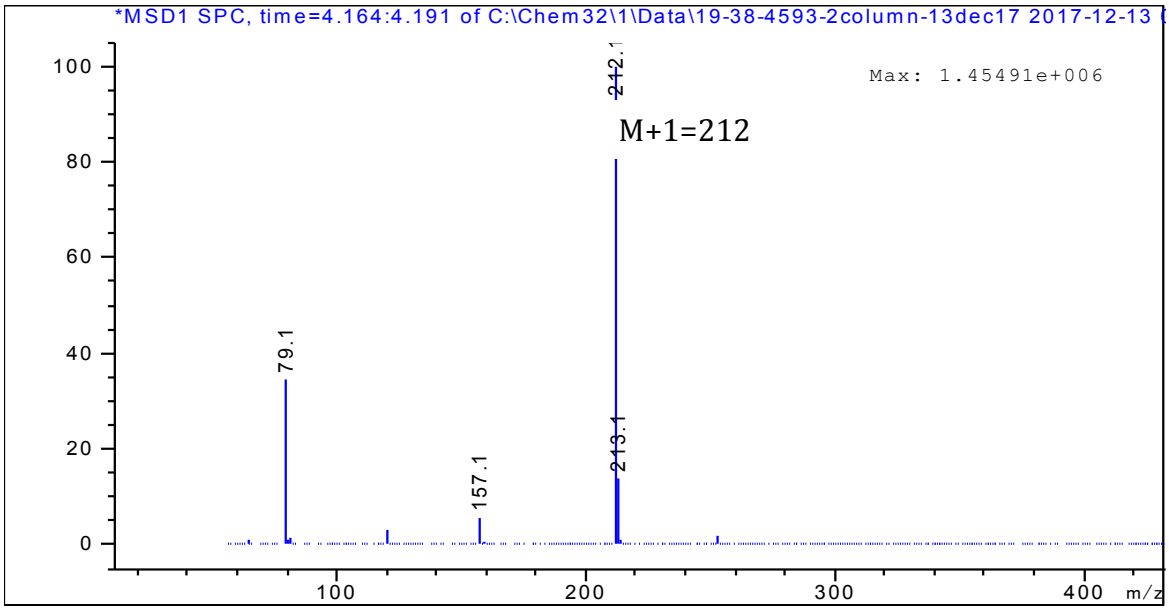
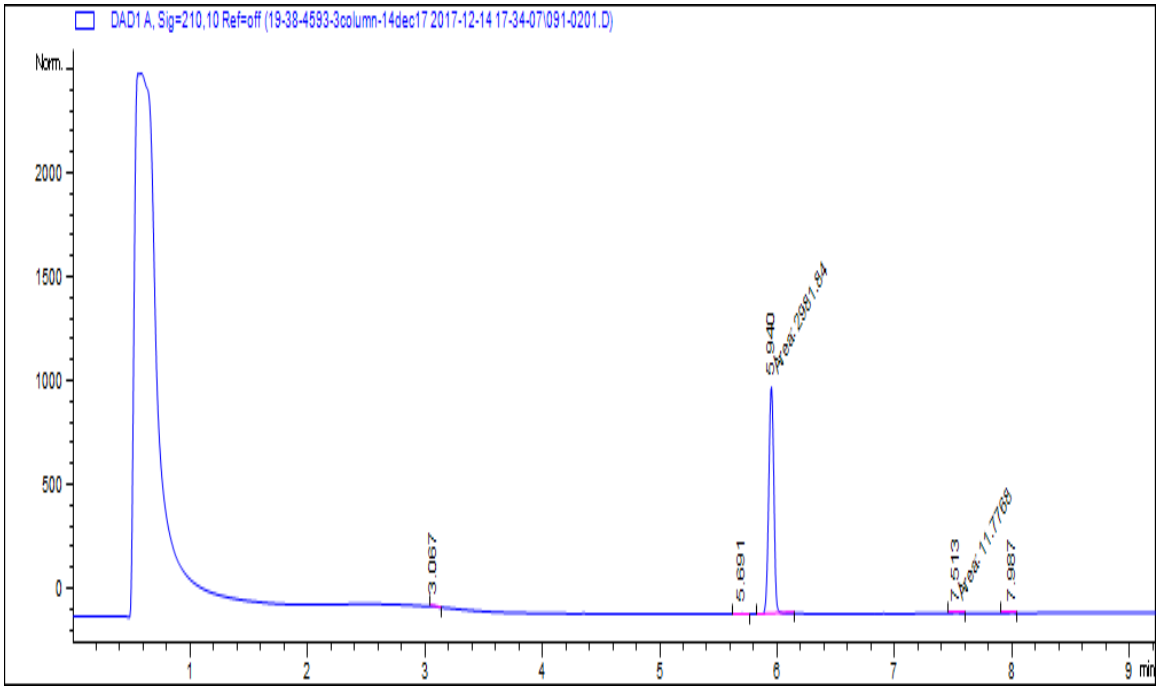


Figure SI 11. LC-MS chromatogram and mass spectrum for SM11. Molecular weight is 211.233 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

A



#	Retention time (minutes)	Area	Area%
1	3.1	5.1	0.2
2	5.7	7.3	0.2
3	5.9	2981.8	98.1
4	7.5	11.8	0.4
5	8.0	6.7	0.2
6	12.3	25.7	0.8

B

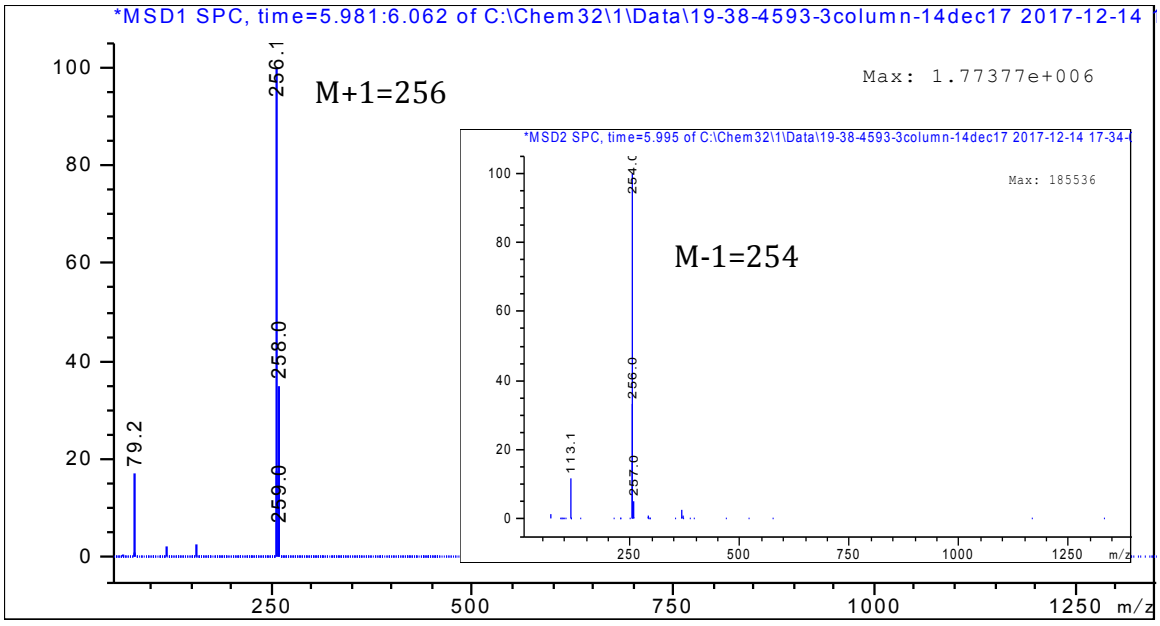
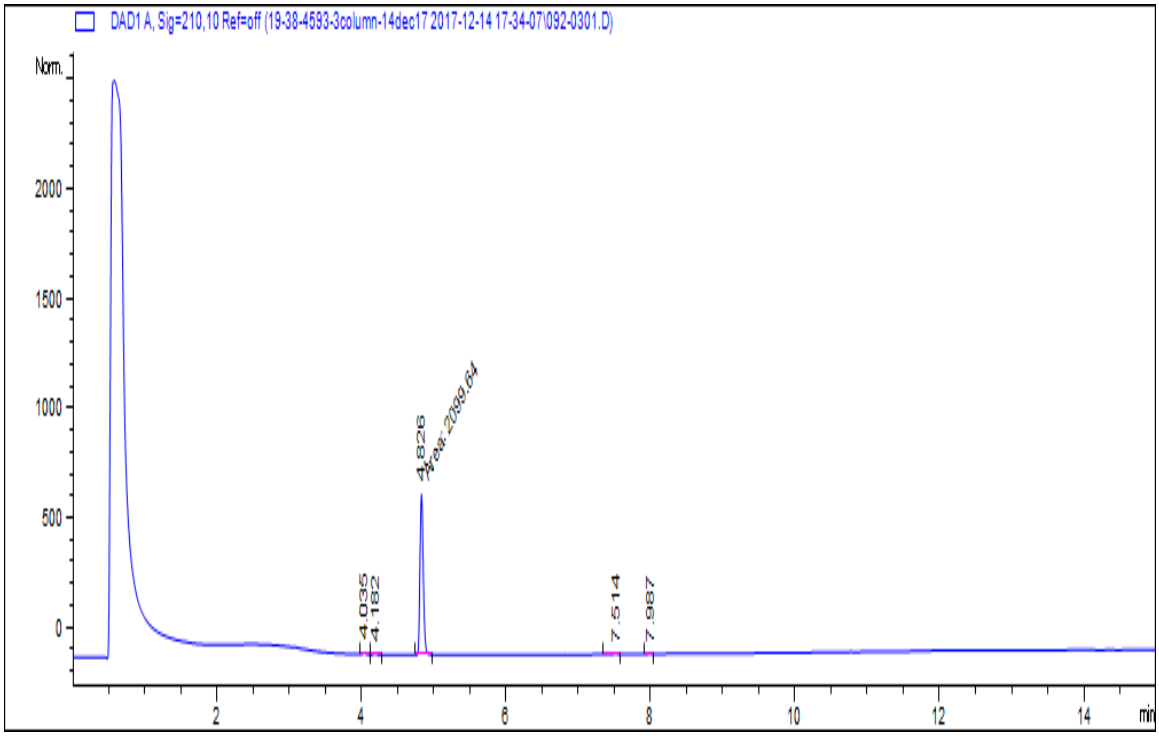


Figure SI 12. LC-MS chromatogram and mass spectrum for SM12. Molecular weight is 292.163 g/mol (m/z = 255). (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

A



#	Retention time (minutes)	Area	Area%
1	4.0	18.1	0.8
2	4.2	15.4	0.7
3	4.8	2099.6	97.5
4	7.5	13.5	0.6
5	8.0	6.5	0.3

B

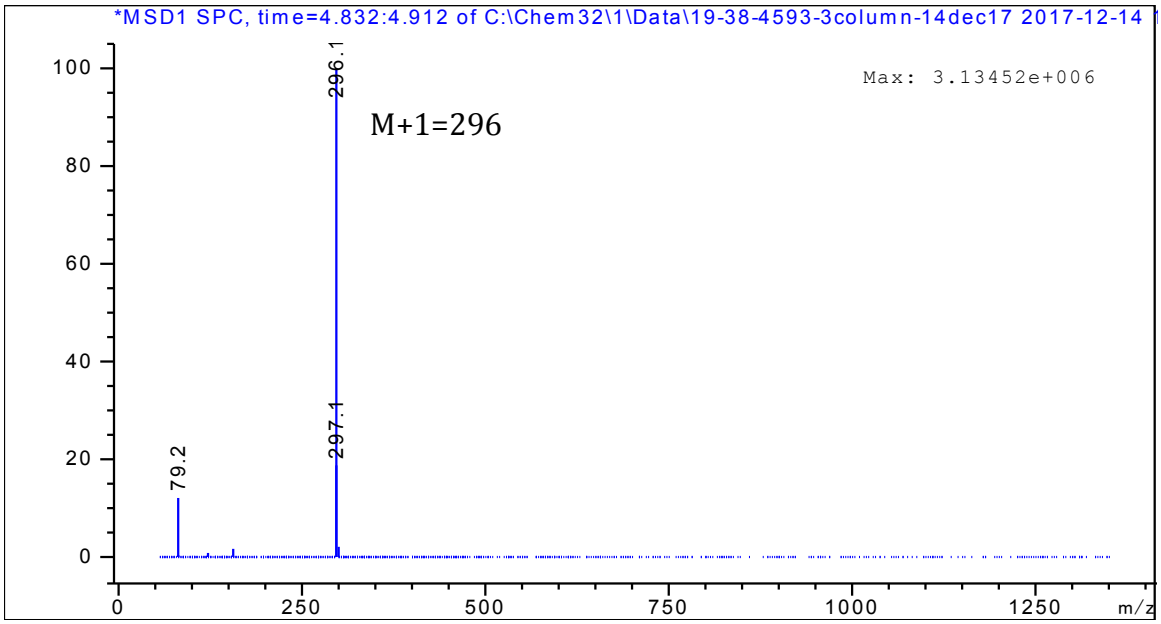
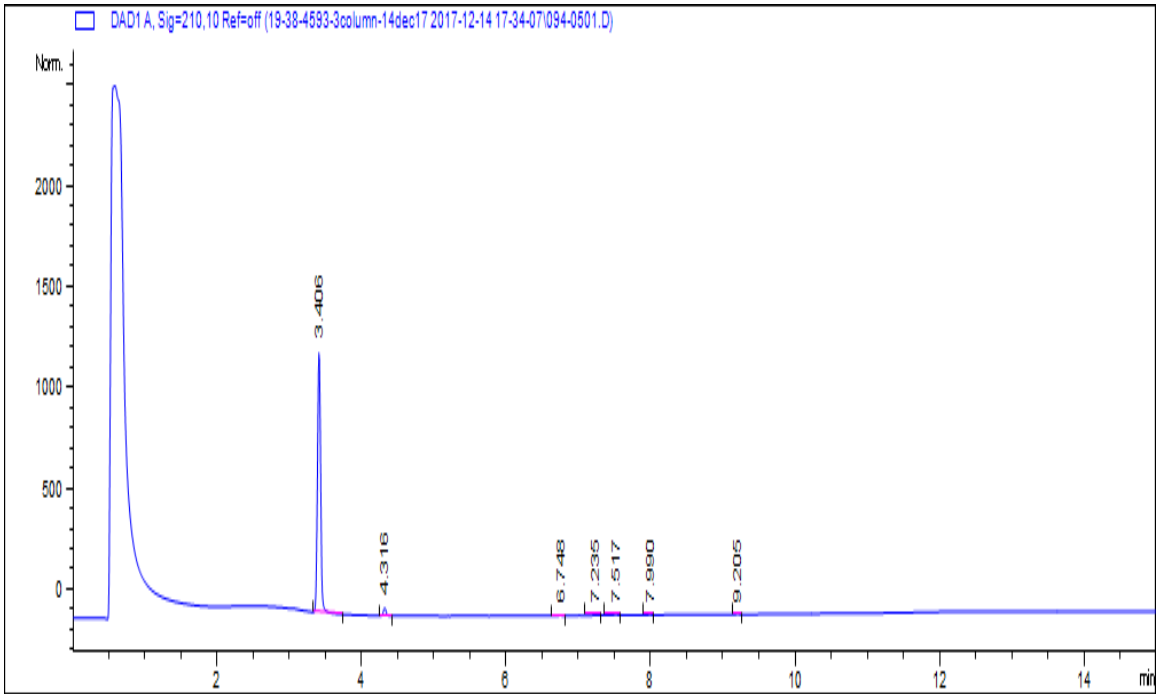


Figure SI 13. LC-MS chromatogram and mass spectrum for SM13. Molecular weight is 295.336 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

A



#	Retention time (minutes)	Area	Area%
1	3.4	3561.5	96.5
2	4.3	90.1	2.4
3	6.7	7.5	0.2
4	7.2	5.9	0.2
5	7.5	14.3	0.4
6	8.0	6.6	0.2
7	9.2	5.3	0.1

B

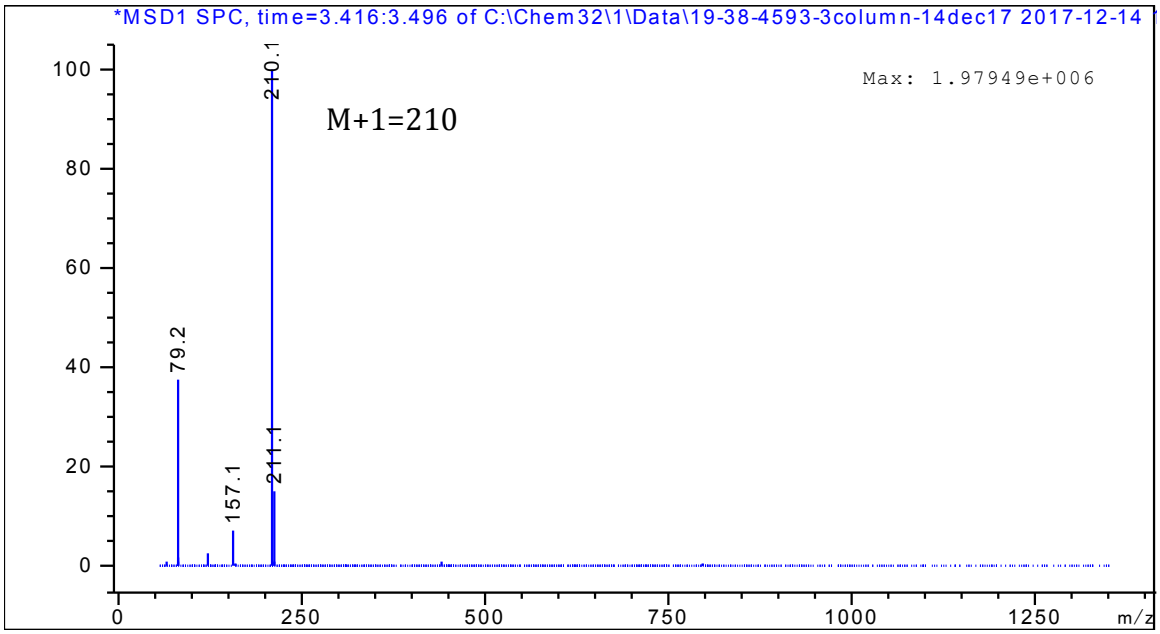
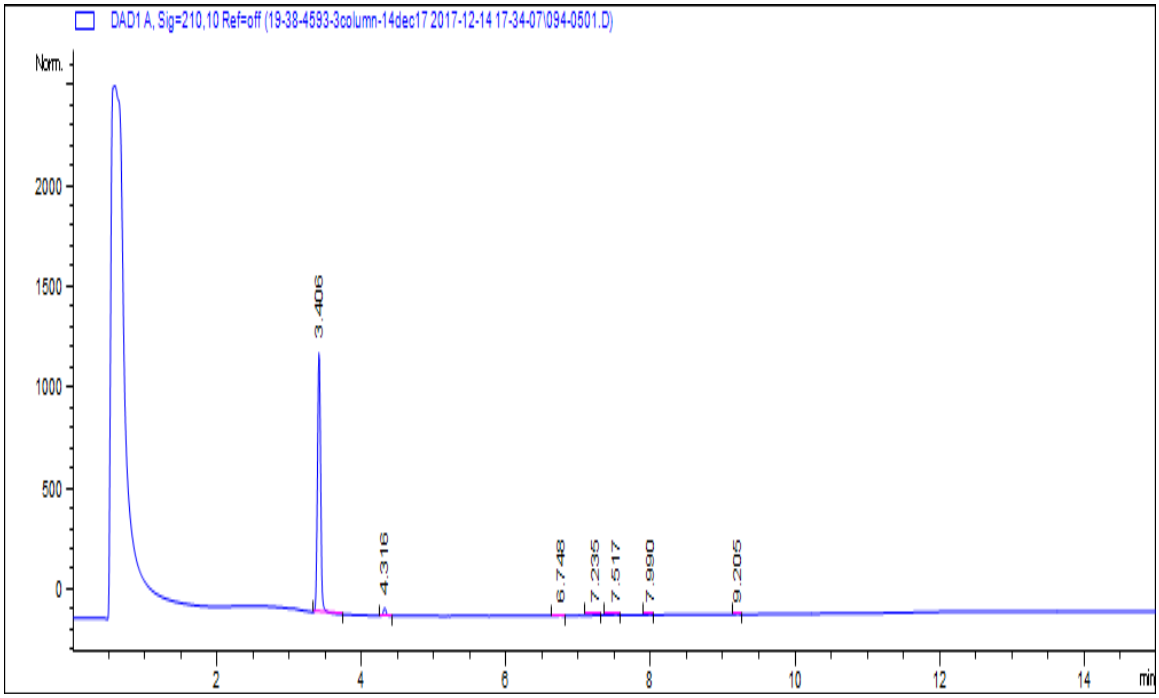


Figure SI 14. LC-MS chromatogram and mass spectrum for SM14. Molecular weight is 209.247 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

A



#	Retention time (minutes)	Area	Area%
1	4.4	2822.7	98.7
2	5.5	15.6	0.5
3	5.8	5.5	0.9
4	7.5	9.4	1.2
5	8.0	6.7	1.0

B

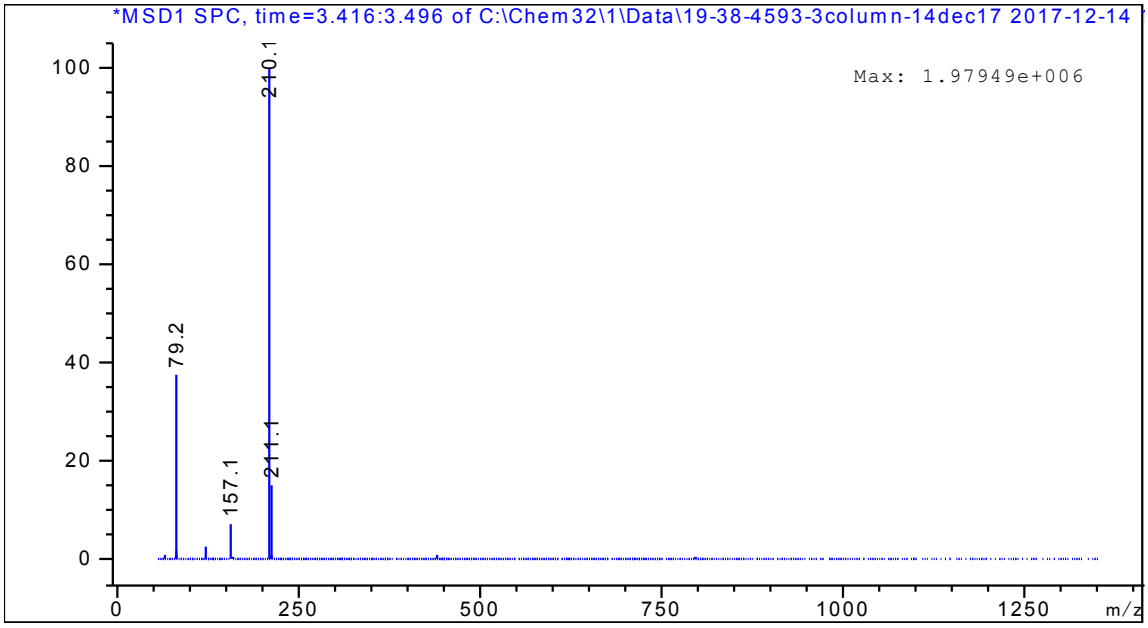
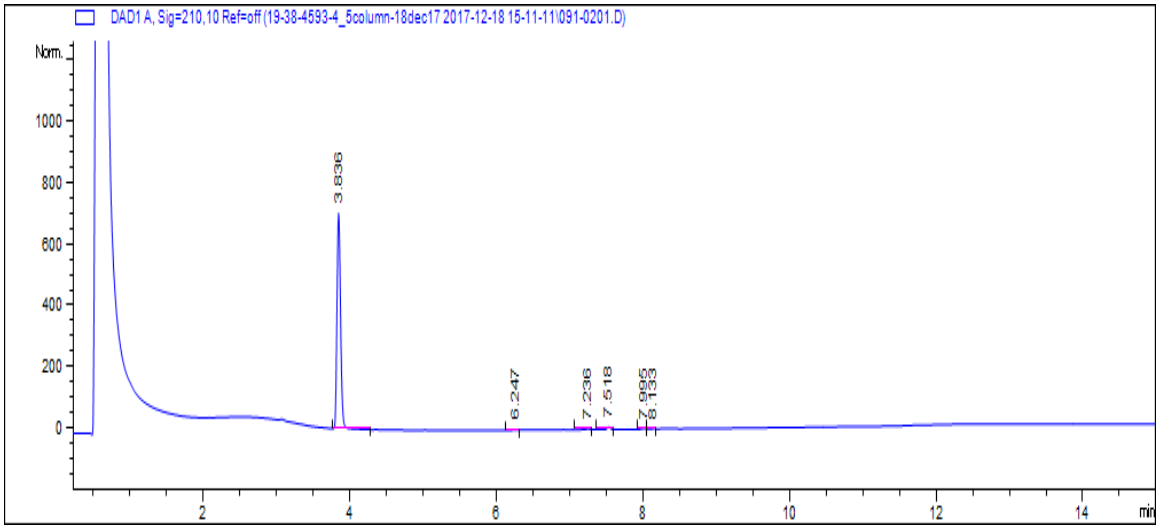


Figure SI 15. LC-MS chromatogram and mass spectrum for SM15. Molecular weight is 210.231 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

A



#	Retention time (minutes)	Area	Area%
1	3.8	2375.8	97.7
2	6.2	5.3	0.2
3	7.2	9.6	0.4
4	7.5	23.9	1.0
5	8.0	10.2	0.4
6	8.1	6.1	0.3

B

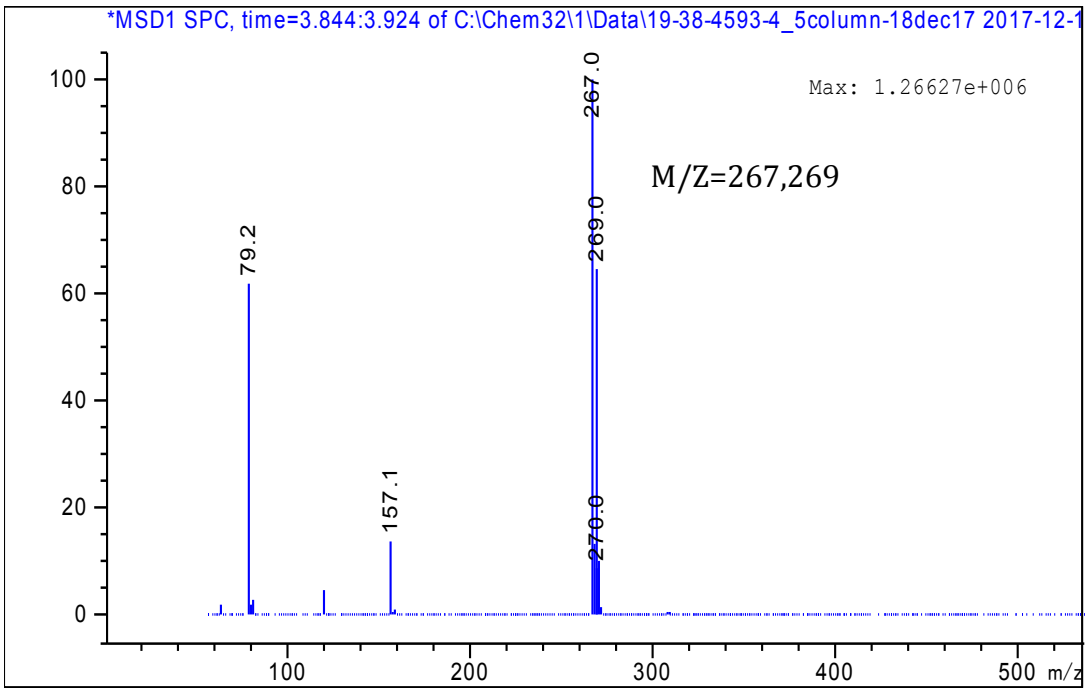
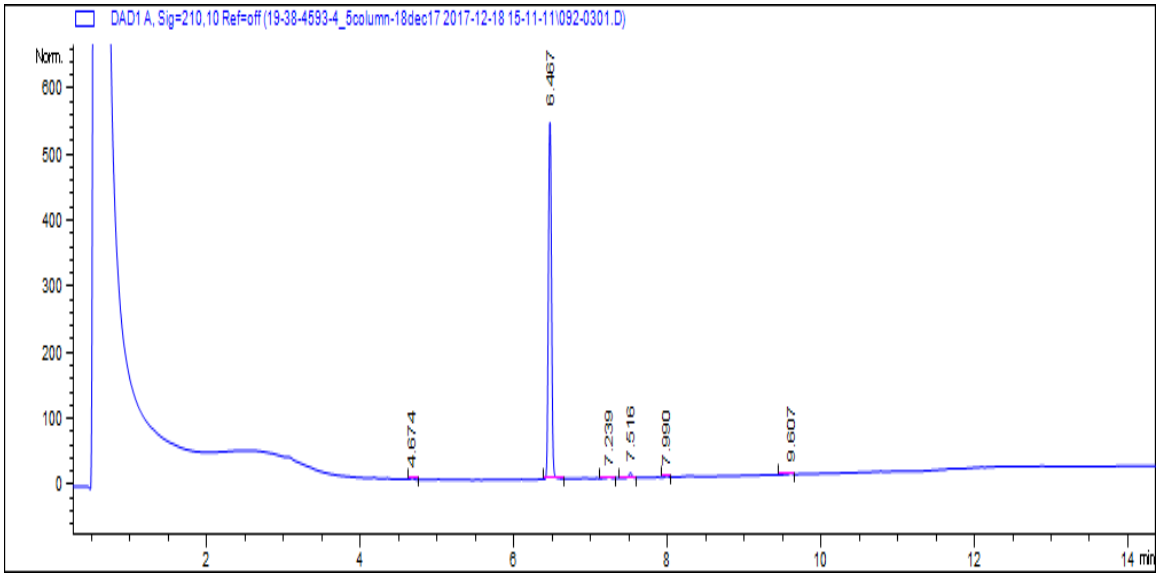


Figure SI 16. LC-MS chromatogram and mass spectrum for SM16. Molecular weight is 267.111 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

A



#	Retention time (minutes)	Area	Area%
1	4.7	5.3	0.3
2	6.5	1541.6	97.0
3	7.2	7.3	0.5
4	7.5	22.5	1.4
5	8.0	6.4	0.4
6	9.6	6.3	0.4

B

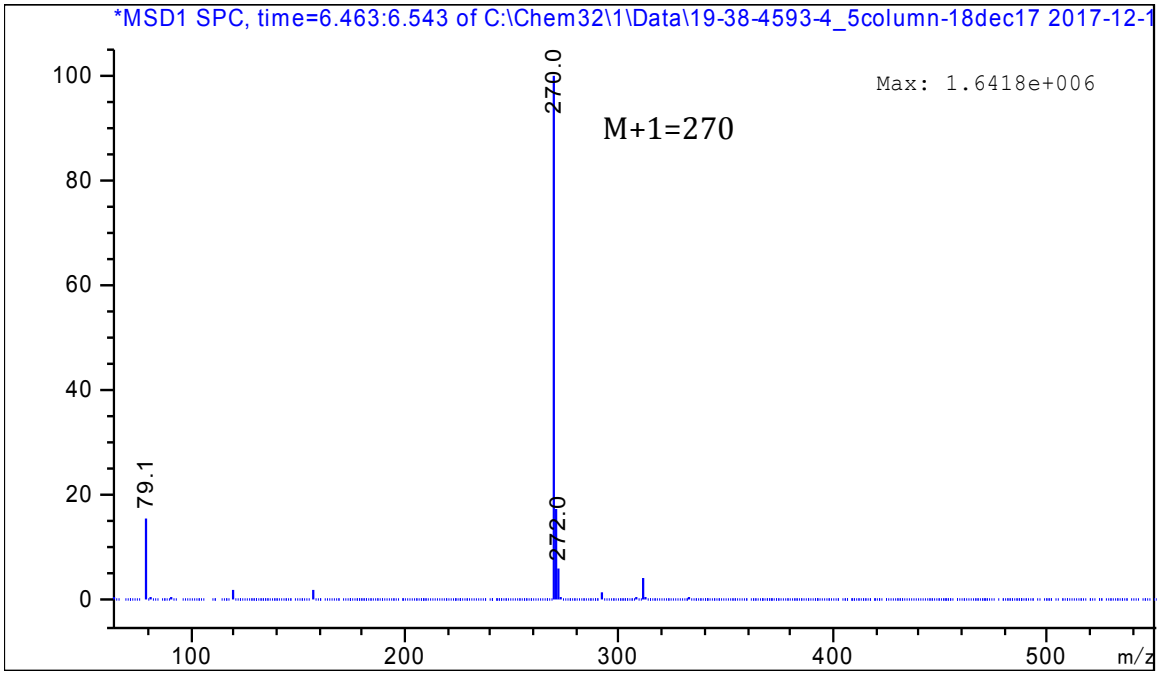
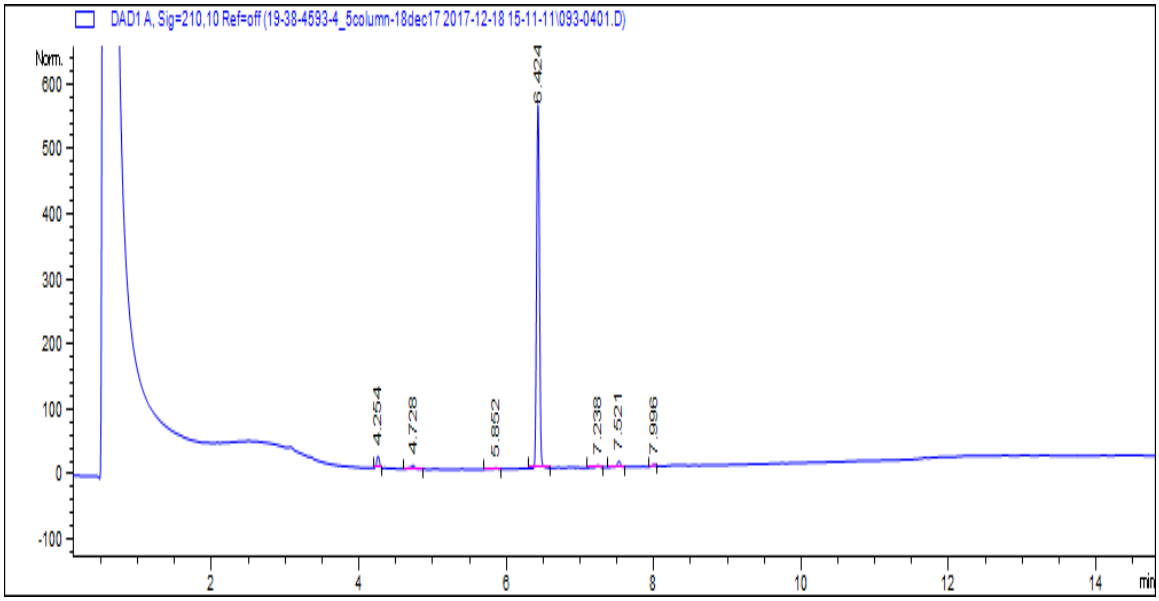


Figure SI 17. LC-MS chromatogram and mass spectrum for SM17. Molecular weight is 269.322 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

A



#	Retention time (minutes)	Area	Area%
1	4.3	47.8	2.9
2	4.7	22.5	1.4
3	5.9	5.7	0.3
4	6.4	1538.3	92.6
5	7.2	10.7	0.6
6	7.5	28.5	1.7
7	8.0	7.8	0.5

B

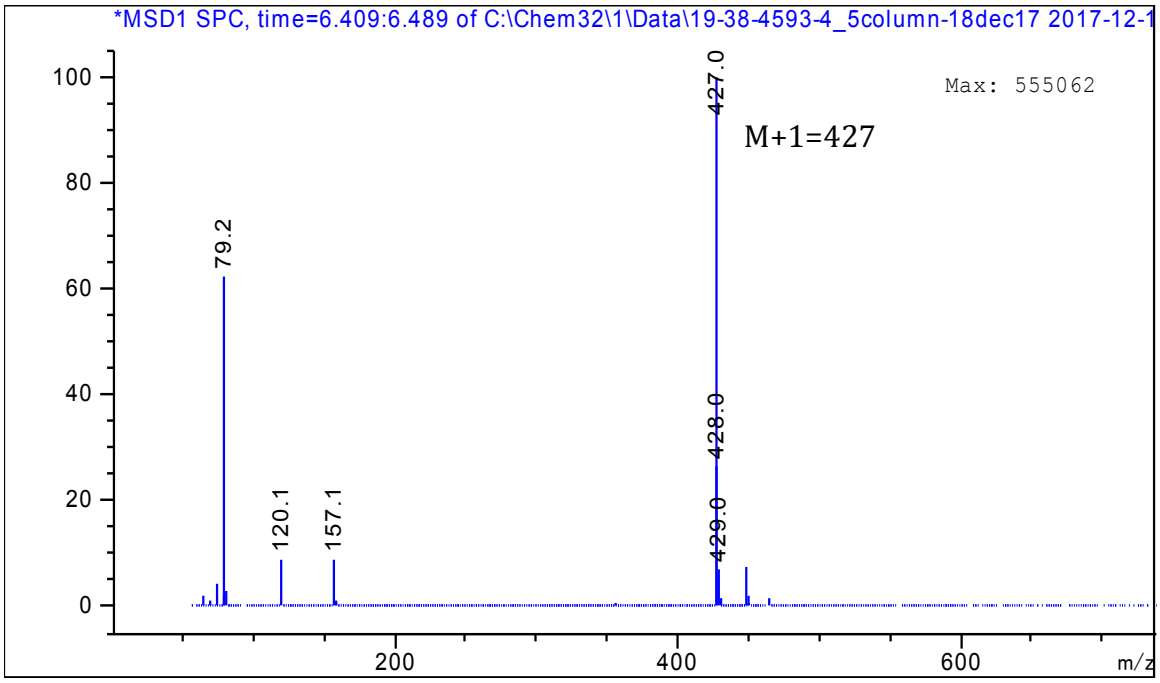
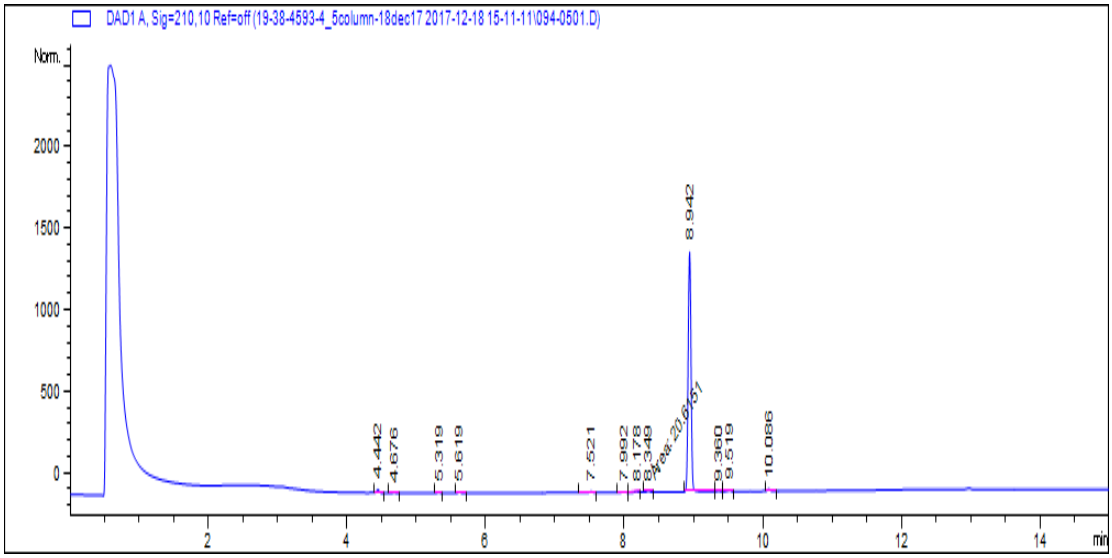


Figure SI 18. LC-MS chromatogram and mass spectrum for SM18. Molecular weight is 426.439 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

A



#	Retention time (minutes)	Area	Area%
1	4.4	51.6	1.3
2	4.7	5.7	0.1
3	5.3	14.3	0.3
4	5.6	17.8	0.4
5	7.5	17.4	0.4
6	8.0	8.2	0.2
7	8.2	14.1	0.3
8	8.3	20.6	0.5
9	8.9	3893.8	94.5
10	9.4	15.4	0.4
11	9.5	26.1	0.6
12	10.1	37.1	0.9

B

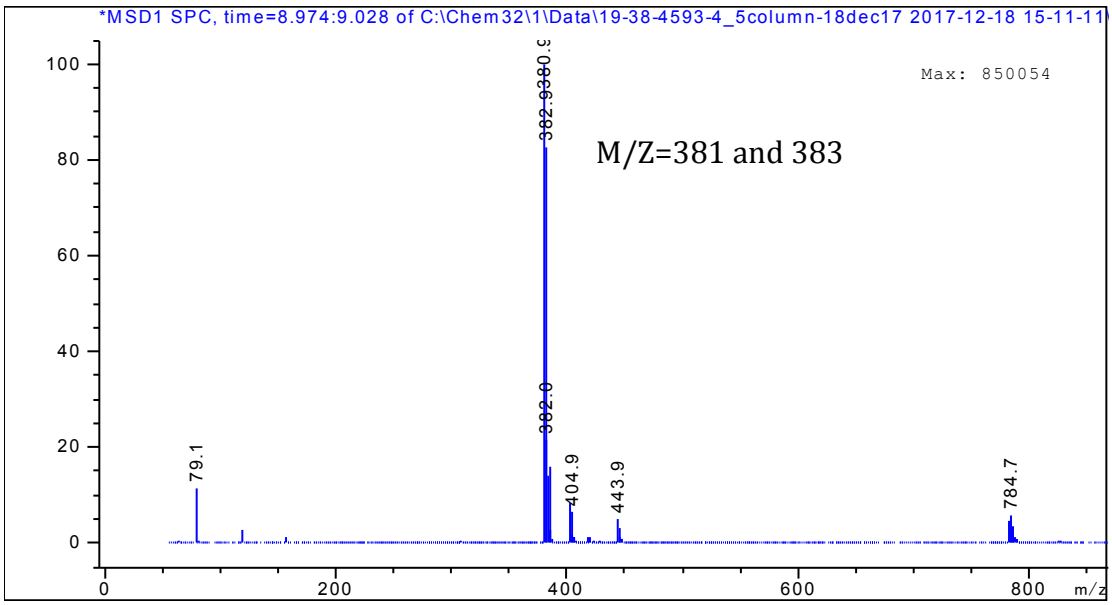
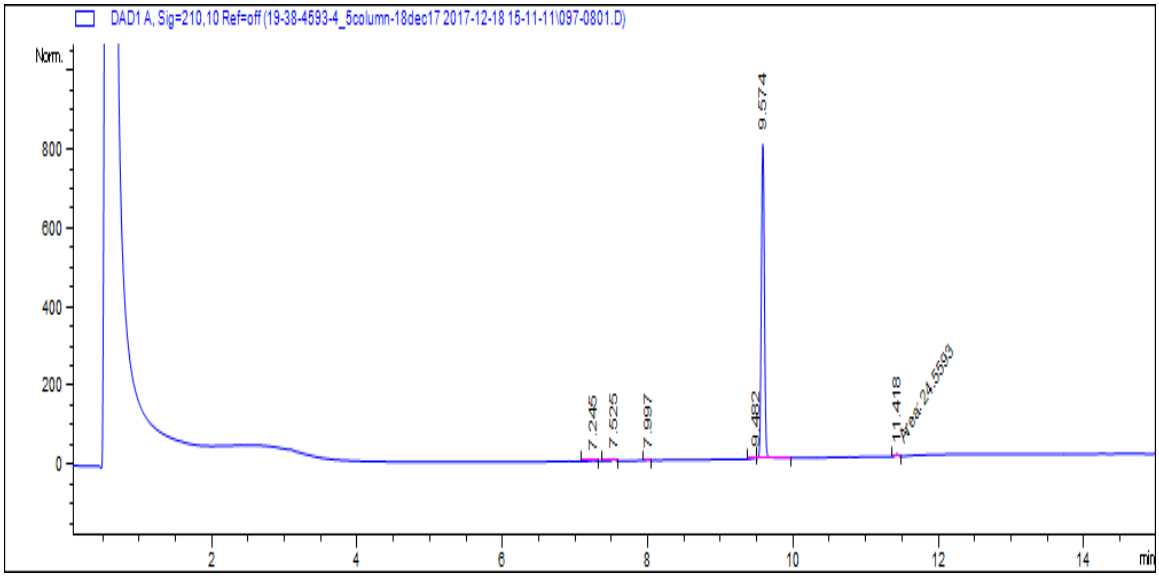


Figure SI 19. LC-MS chromatogram and mass spectrum for SM19. Molecular weight is 381.276 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

A



#	Retention time (minutes)	Area	Area%
1	7.2	5.6	0.2
2	7.5	16.4	0.7
3	8.0	7.0	0.3
4	9.5	15.4	0.6
5	9.6	2376.4	97.2
6	11.4	24.6	1.0

B

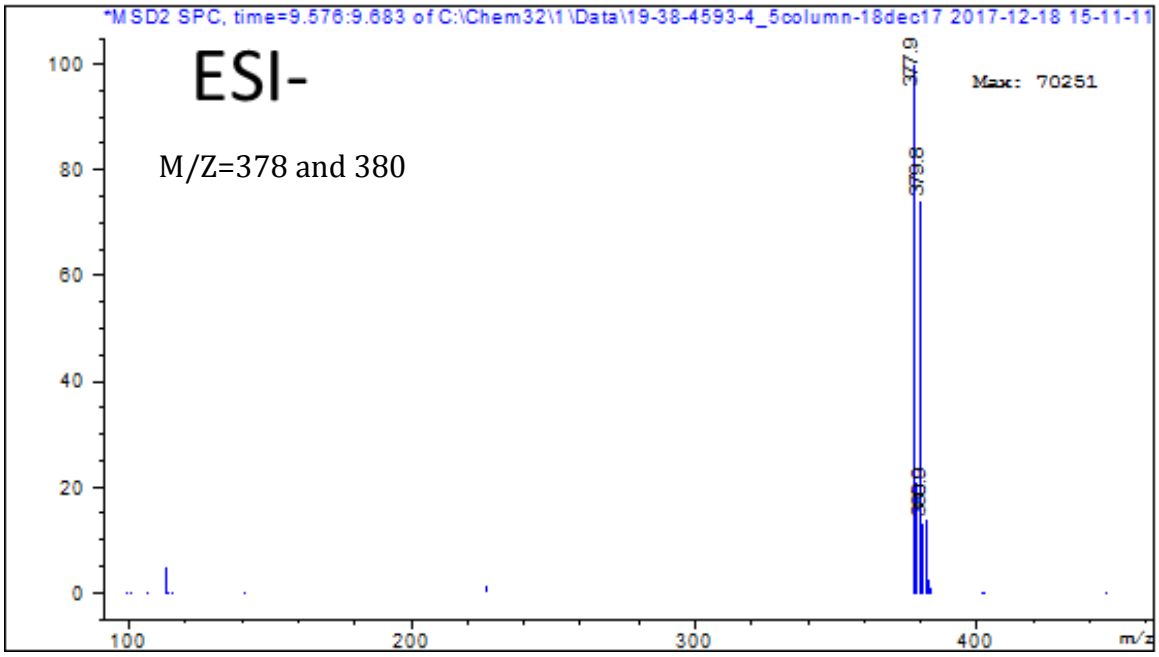
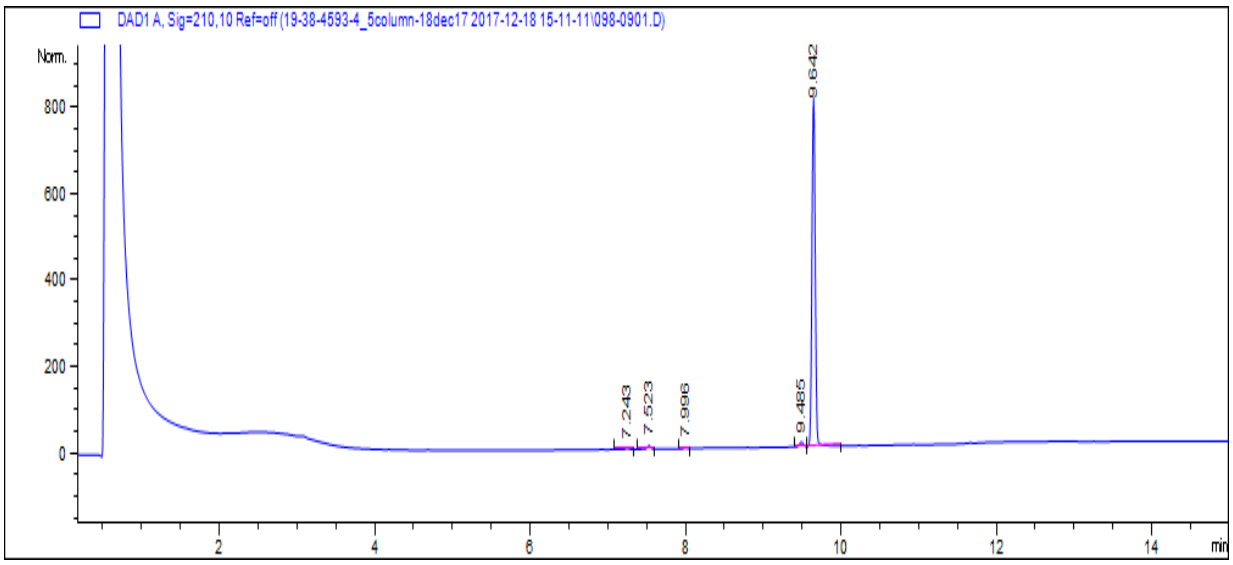


Figure SI 20. LC-MS chromatogram mass spectrum for SM20. Molecular weight is 380.245 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

A



#	Retention time (minutes)	Area	Area%
1	7.2	9.1	0.4
2	7.5	23.6	1.0
3	8.0	6.4	0.2
4	9.5	39.9	1.6
5	9.6	2381.2	96.8

B

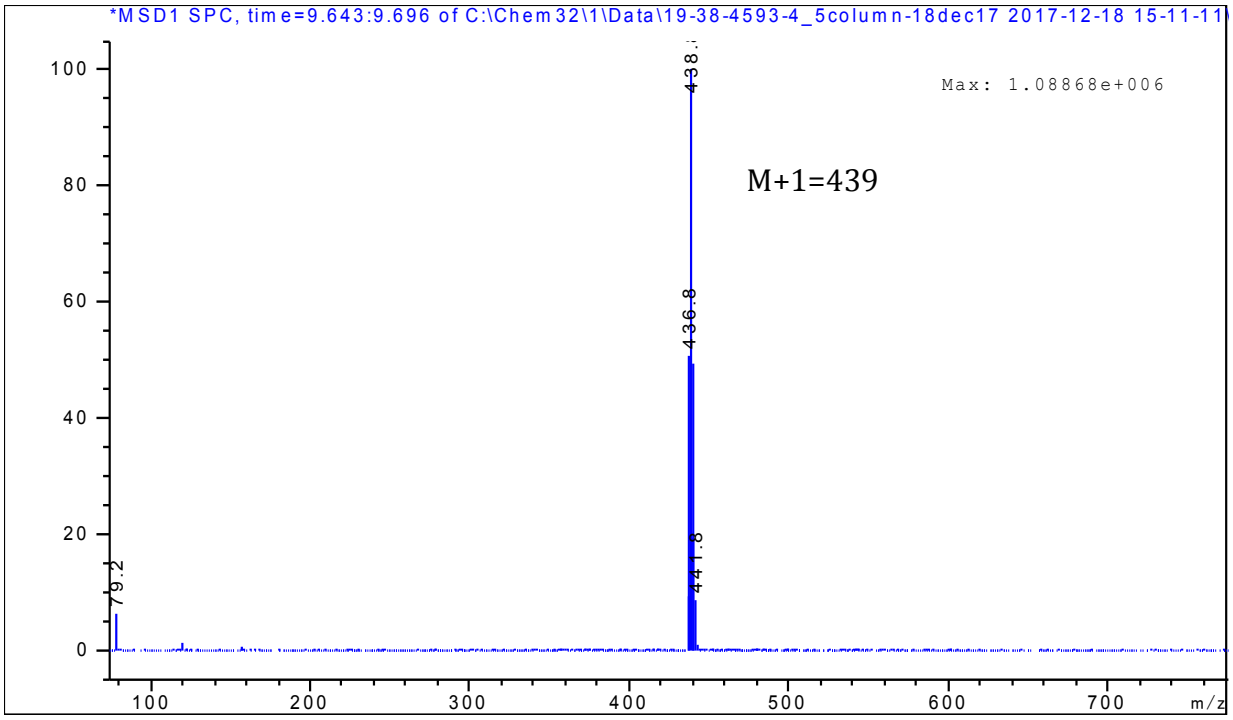
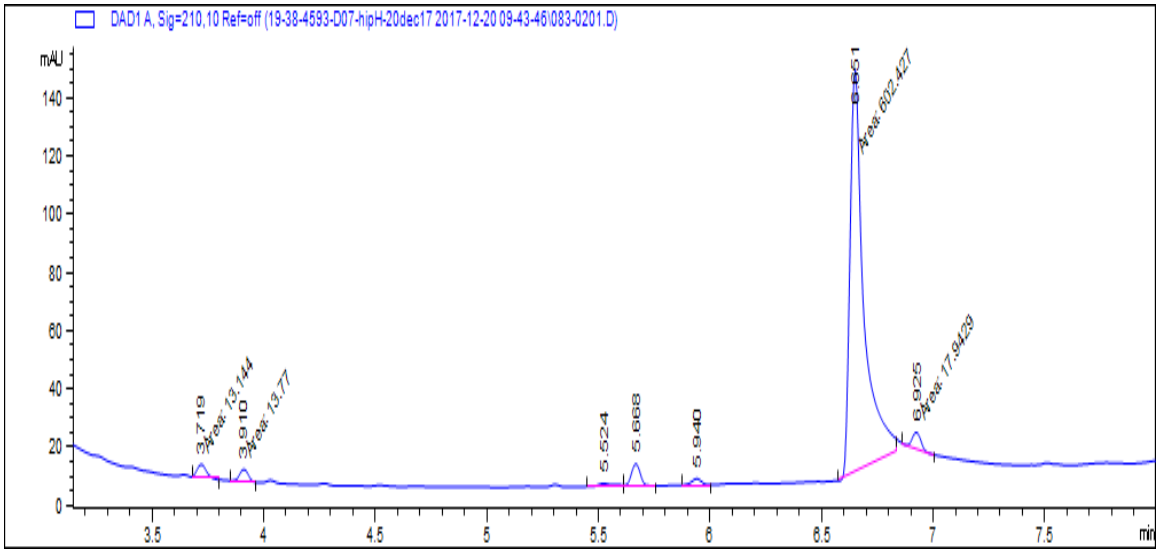


Figure SI 21. LC-MS chromatogram and mass spectrum for SM21. Molecular weight is 438.092 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

A



#	Retention time (minutes)	Area	Area%
1	3.7	13.1	1.9
2	3.9	13.8	2.0
3	5.5	8.0	1.2
4	5.7	24.9	3.6
5	5.9	12.4	1.8
6	6.7	602.4	87.0
7	6.9	17.9	2.6

B

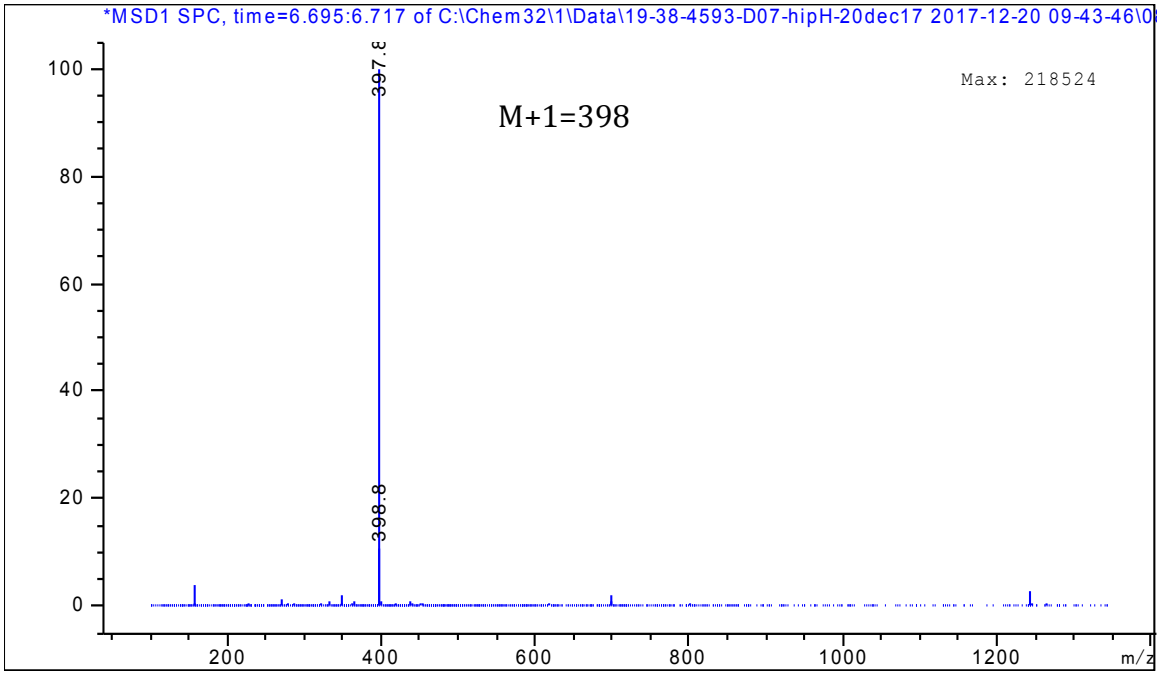
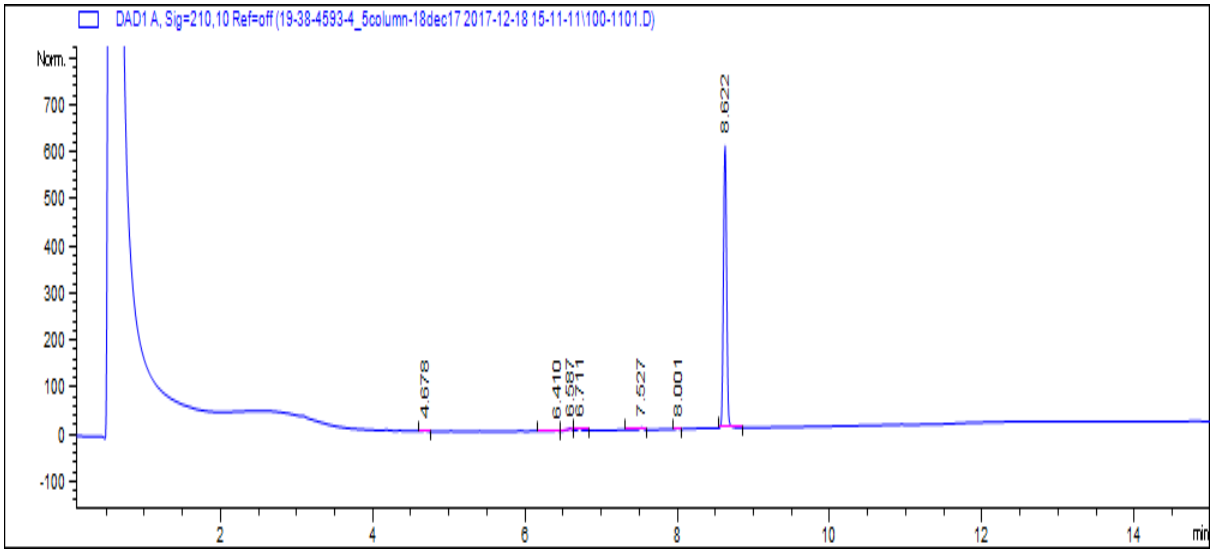


Figure SI 22. LC-MS chromatogram and mass spectrum for SM22. Molecular weight is 396.951 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

A



#	Retention time (minutes)	Area	Area%
1	4.7	5.4	0.3
2	6.4	5.6	0.3
3	6.6	22.7	1.2
4	6.7	18.1	1.0
5	7.5	16.1	0.9
6	8.0	5.6	0.3
7	8.6	1770.7	96.0

B

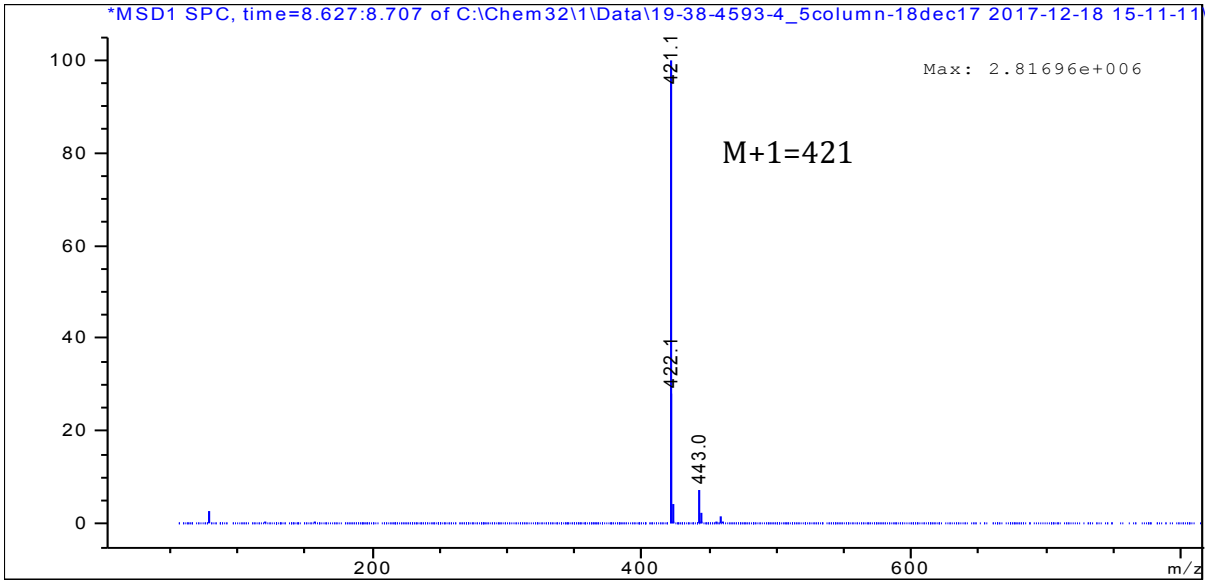
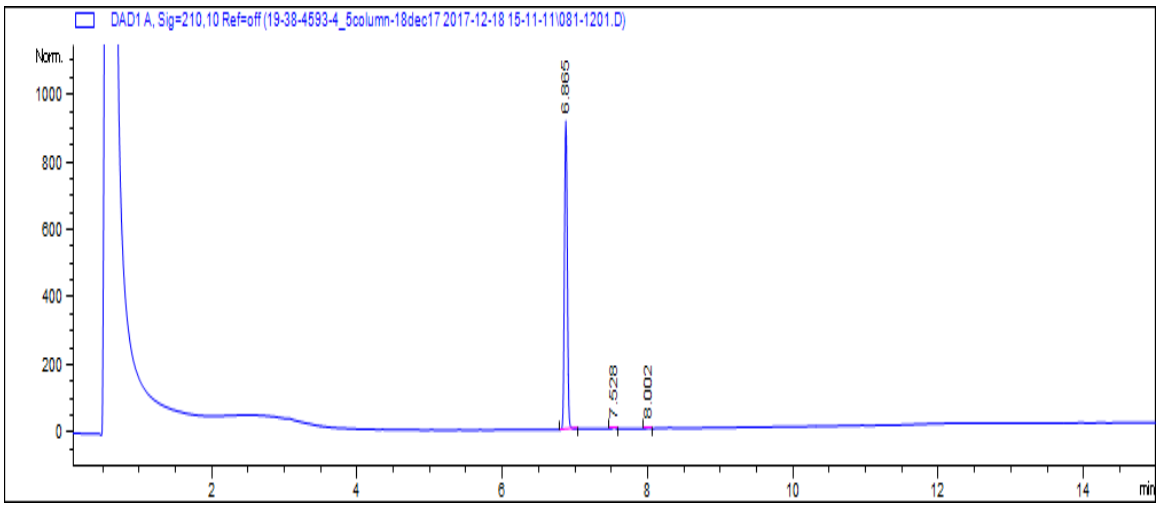


Figure SI 23. LC-MS chromatogram and mass spectrum for SM23. Molecular weight is 420.461 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

A



#	Retention time (minutes)	Area	Area%
1	6.9	2511.1	99.3
2	7.5	11.0	0.4
3	8.0	5.9	0.2

B

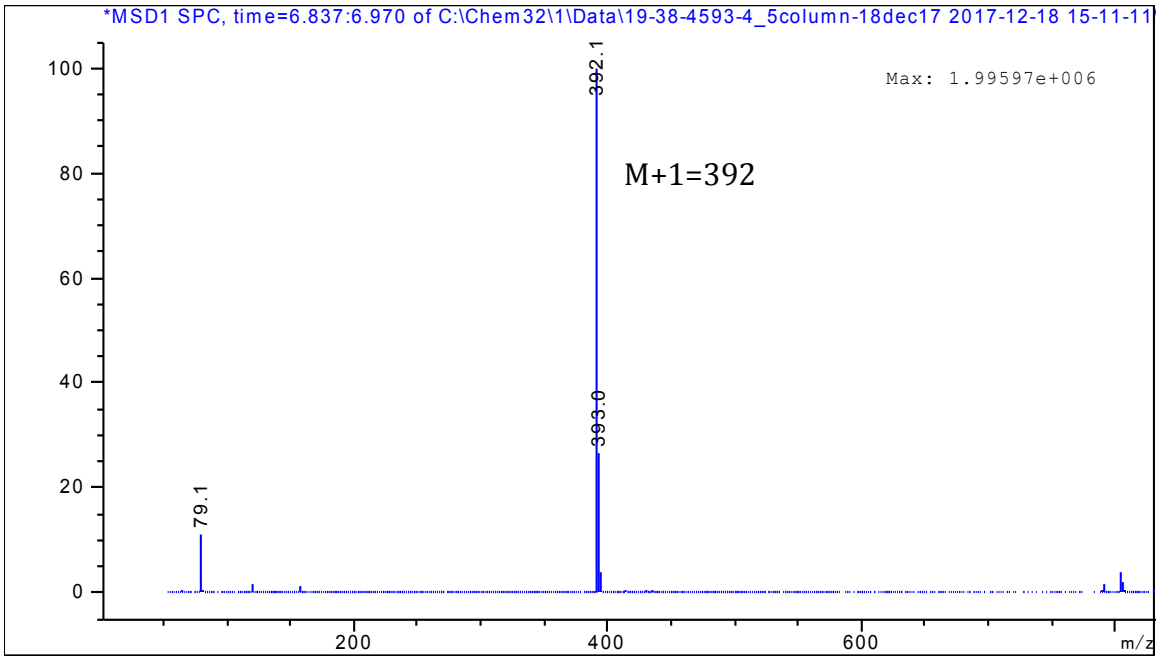


Figure SI 24. LC-MS chromatogram and mass spectrum for SM24. Molecular weight is 391.42 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

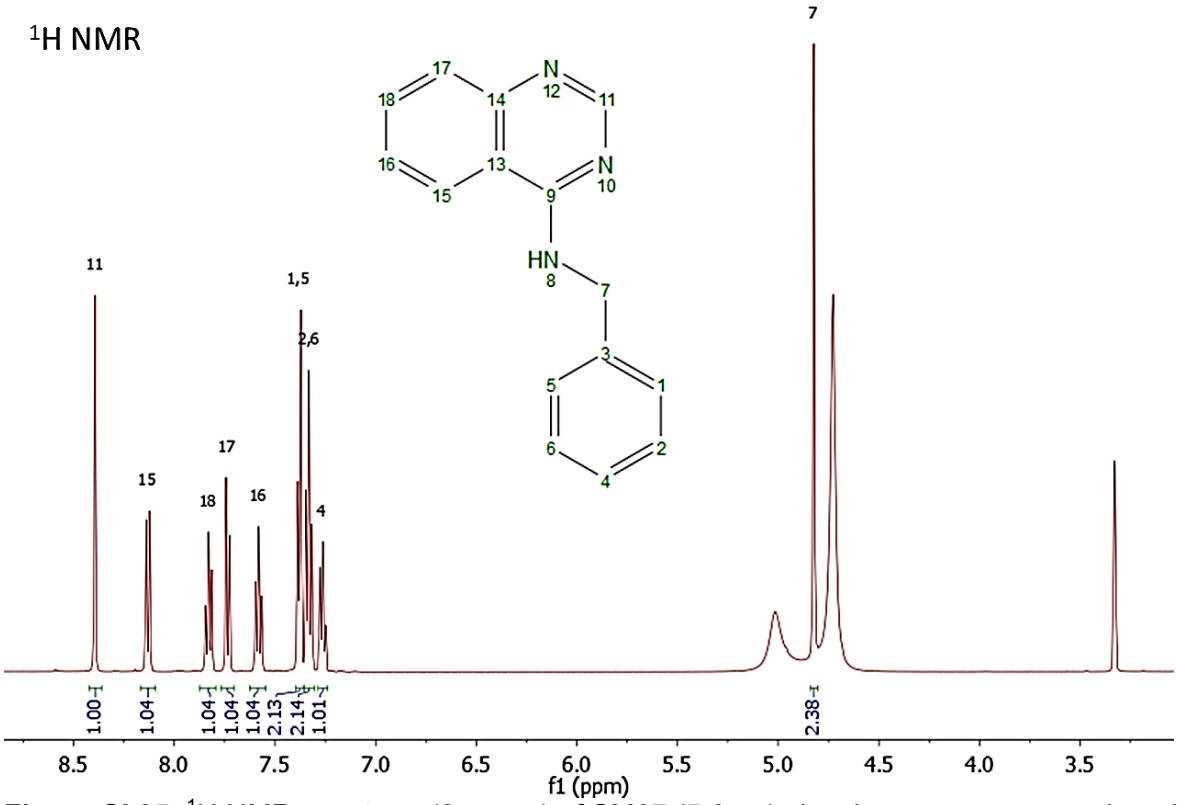


Figure SI 25. ^{1}H NMR spectrum (8 scans) of SM07 (5.8mg) showing resonances assigned to the corresponding protons. The spectrum was acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe^{TM} prodigy at 298 K in 0.6 ml water-d_2/methanol-d_4 (1:2).

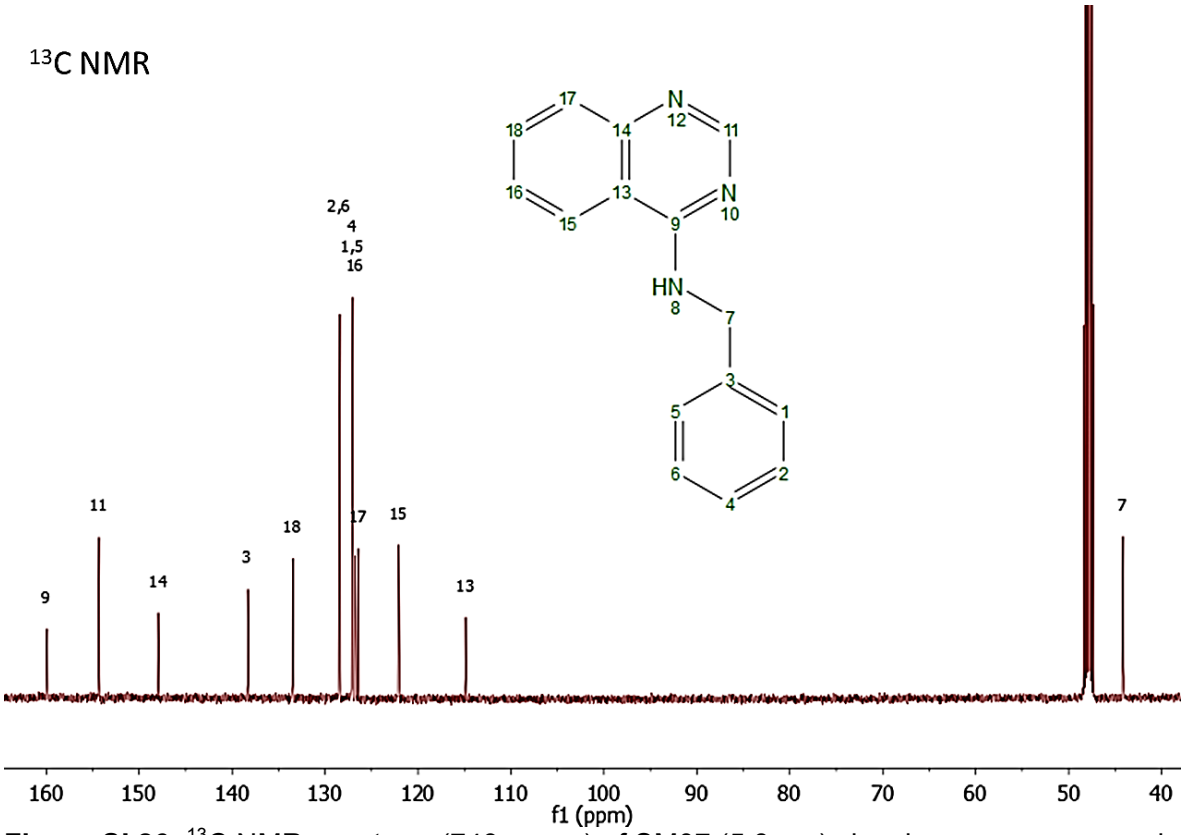


Figure SI 26. ^{13}C NMR spectrum (749 scans) of SM07 (5.8 mg) showing resonances assigned to the corresponding carbon nuclei. The spectrum was acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe^{TM} prodigy at 298 K in 0.6 ml water-d_2/methanol-d_4 (1:2).

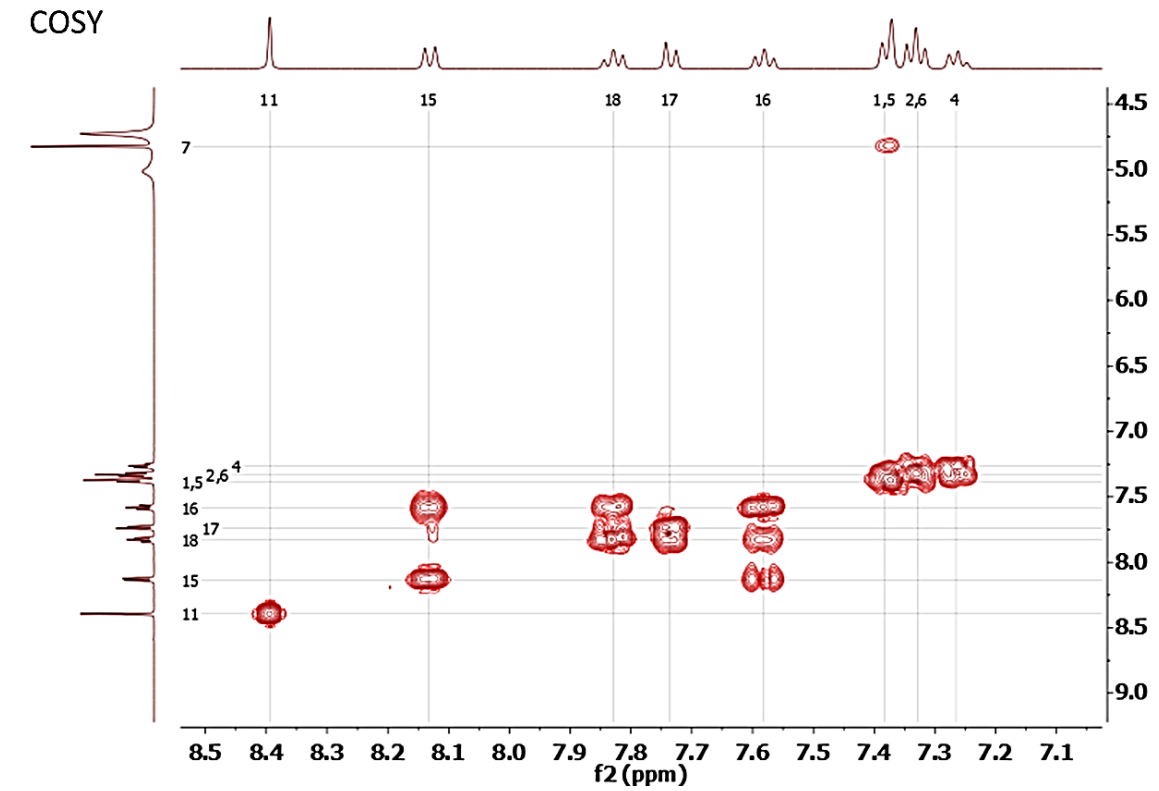


Figure SI 27. A 4 scan Correlated Spectroscopy (COSY) spectrum of SM07 (5.8 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml water-d_2/methanol-d_4 (1:2). Cross-peaks (off-diagonal peaks) corresponds to coupled adjacent protons.

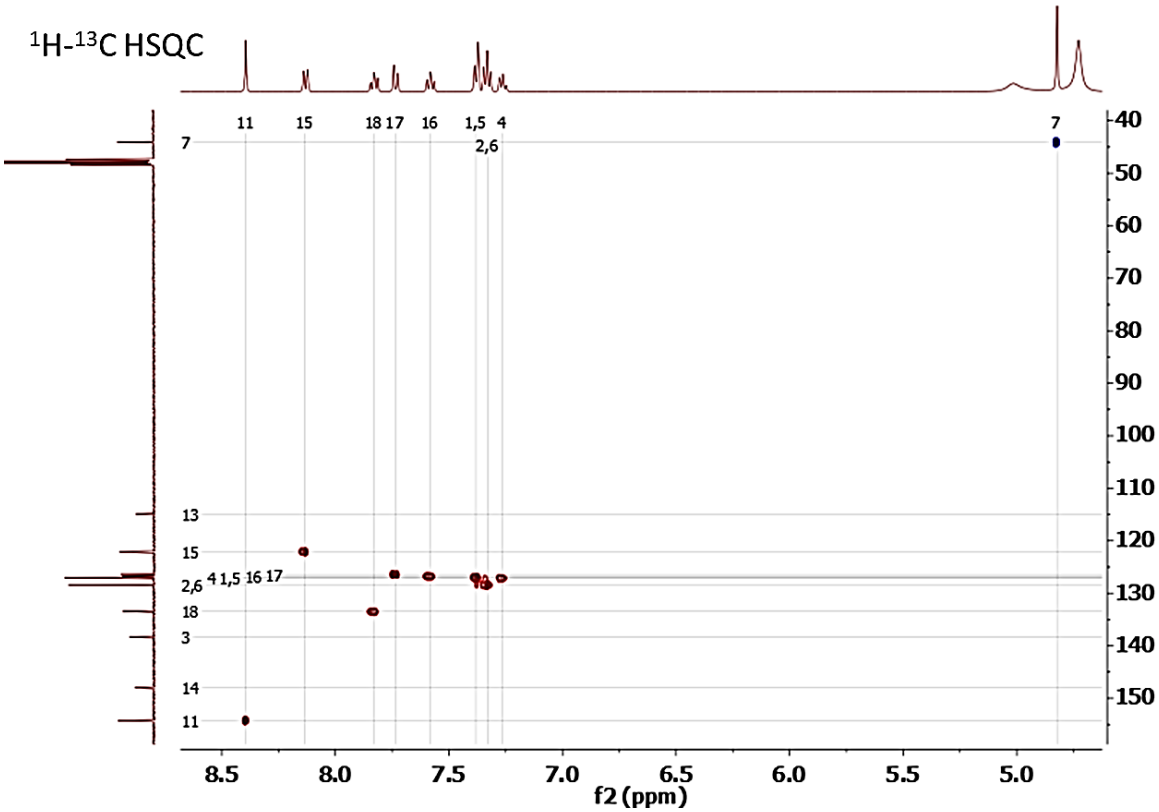


Figure SI 28. A 2 scan ^{1}H-^{13}C HSQC (heteronuclear single quantum correlation) spectrum of SM07 (5.8 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml water-d_2/methanol-d_4 (1:2). Each peak correlates a carbon to its attached proton(s).

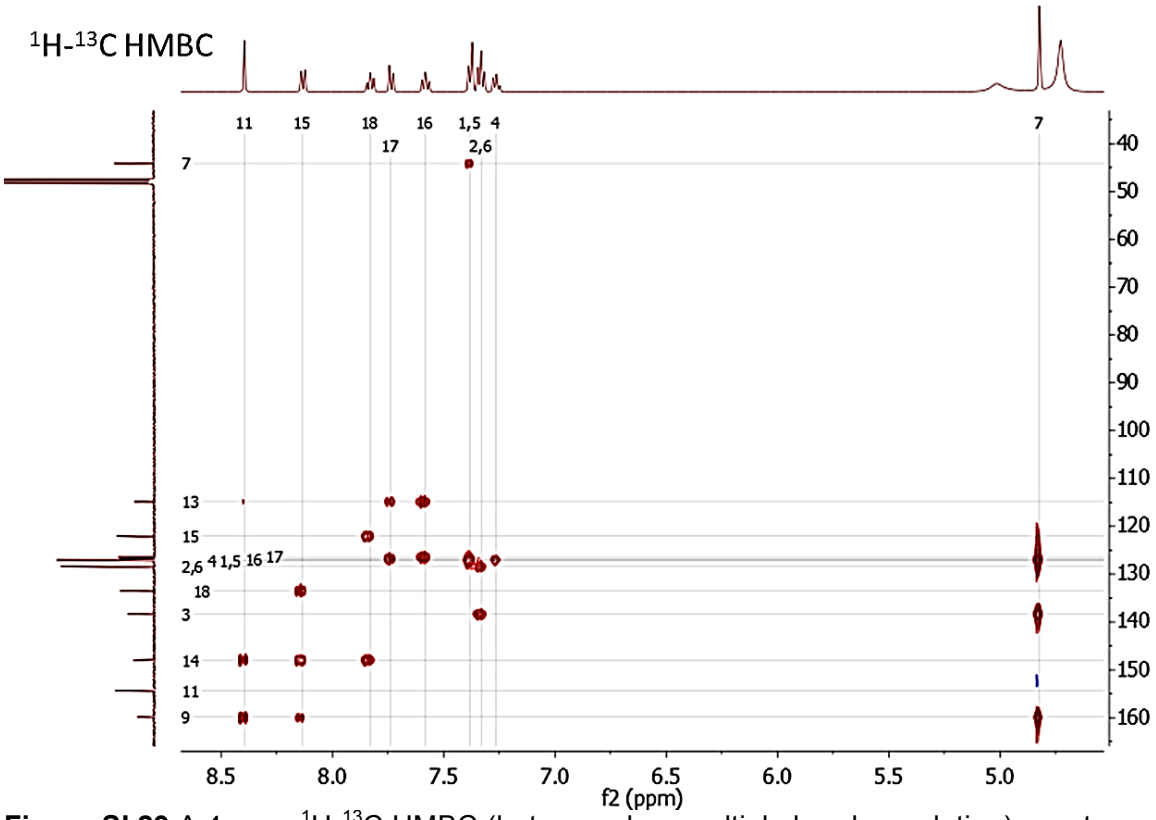


Figure SI 29 A 4 scan ^{1}H-^{13}C HMBC (heteronuclear multiple bond correlation) spectrum of SM07 (5.8 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml water-d_2/methanol-d_4 (1:2). Peaks corresponds to coupling between the proton of interest to neighboring carbons, which are 2 – 4 bonds away.

^{1}H-^{15}N HMBC

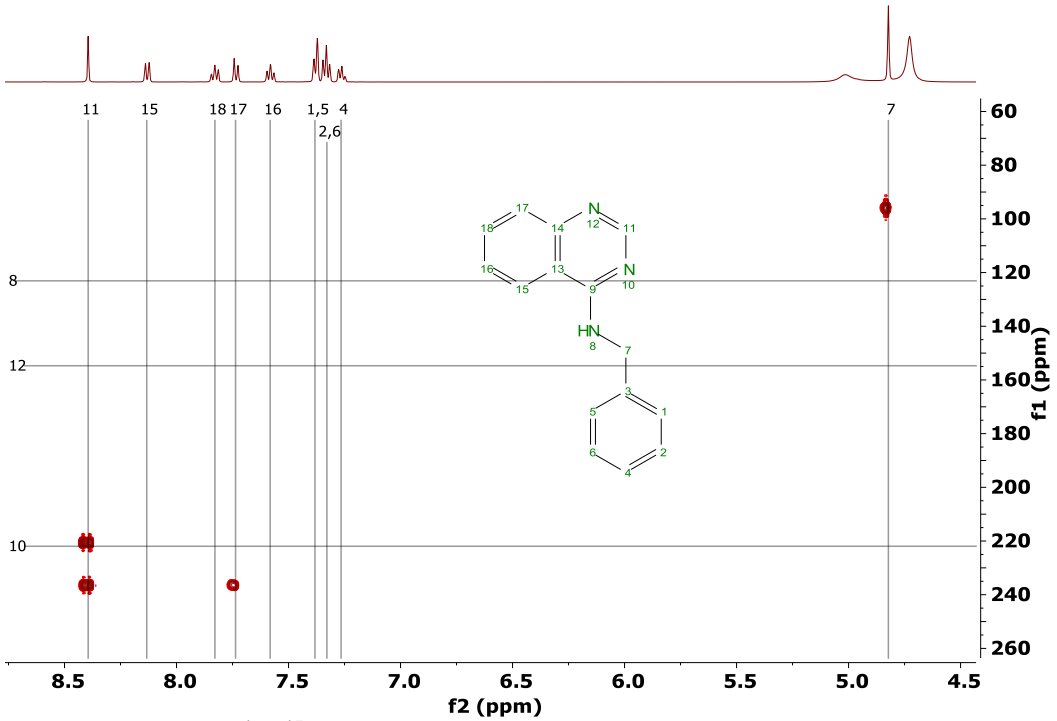


Figure SI 30. A 64 scan ^{1}H-^{15}N HMBC (heteronuclear multiple bond correlation) spectrum of SM07 (5.8 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml water-d_2/methanol-d_4 (1:2). The ^{15}N chemical shifts have been assigned by their correlation to near-by protons. Peaks corresponds to coupling between the proton of interest to neighboring nitrogens, which are 2 – 3 bonds away. No TFA titrated.

^{1}H-^{15}N HMBC

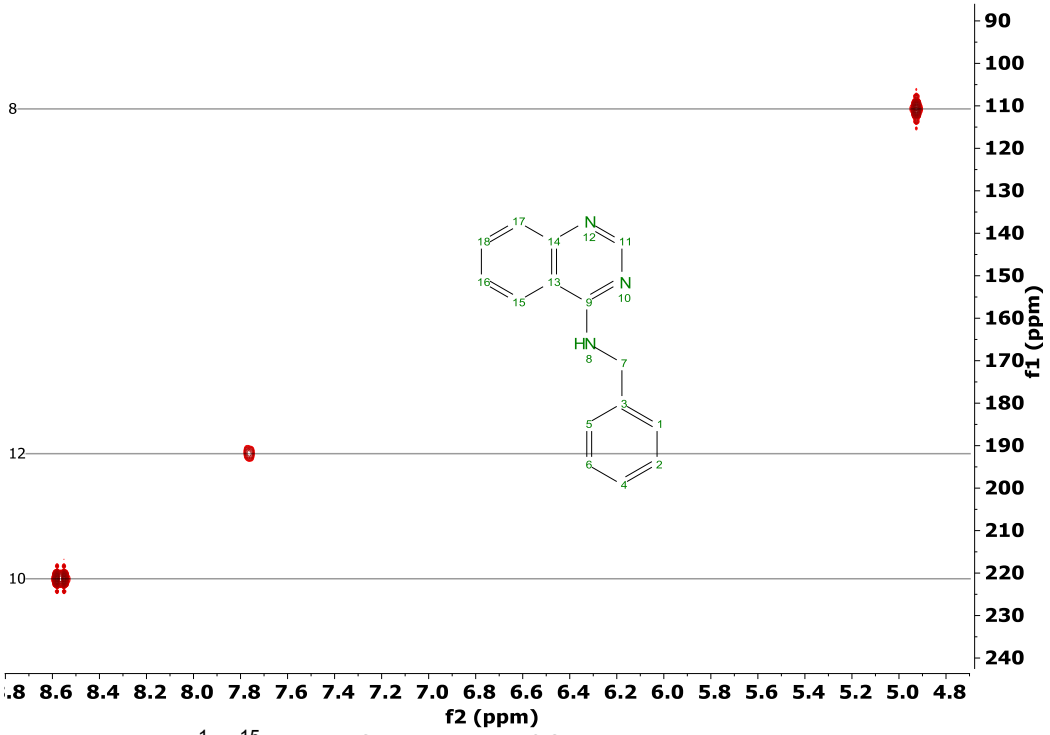


Figure SI 31. A 40 scan ^{1}H-^{15}N HMBC spectrum of SM07 (5.8 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml water-d_2/methanol-d_4 (1:2). The ^{15}N chemical shifts have been assigned by their correlation to near-by protons (not shown). 0.5 equivalents of TFA titrated.

^{1}H-^{15}N HMBC

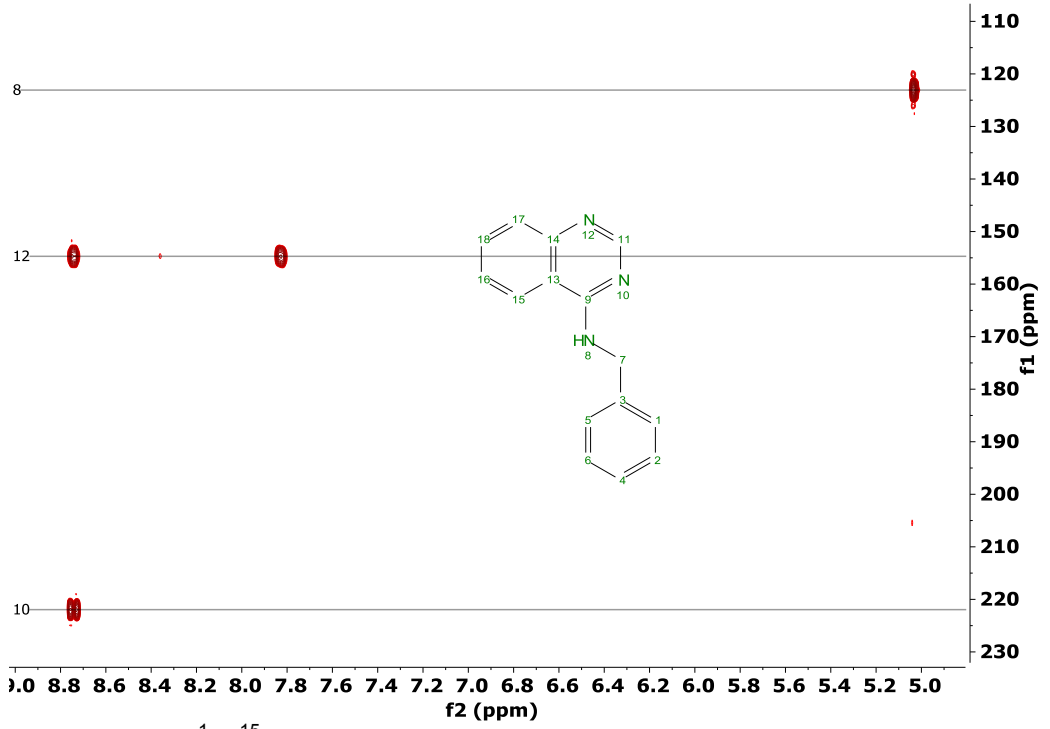


Figure SI 32. A 40 scan ^{1}H-^{15}N HMBC spectrum of SM07 (5.8 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml water-d_2/methanol-d_4 (1:2). The ^{15}N chemical shifts have been assigned by their correlation to near-by protons (not shown). 1.0 equivalents of TFA titrated.

^{1}H-^{15}N HMBC

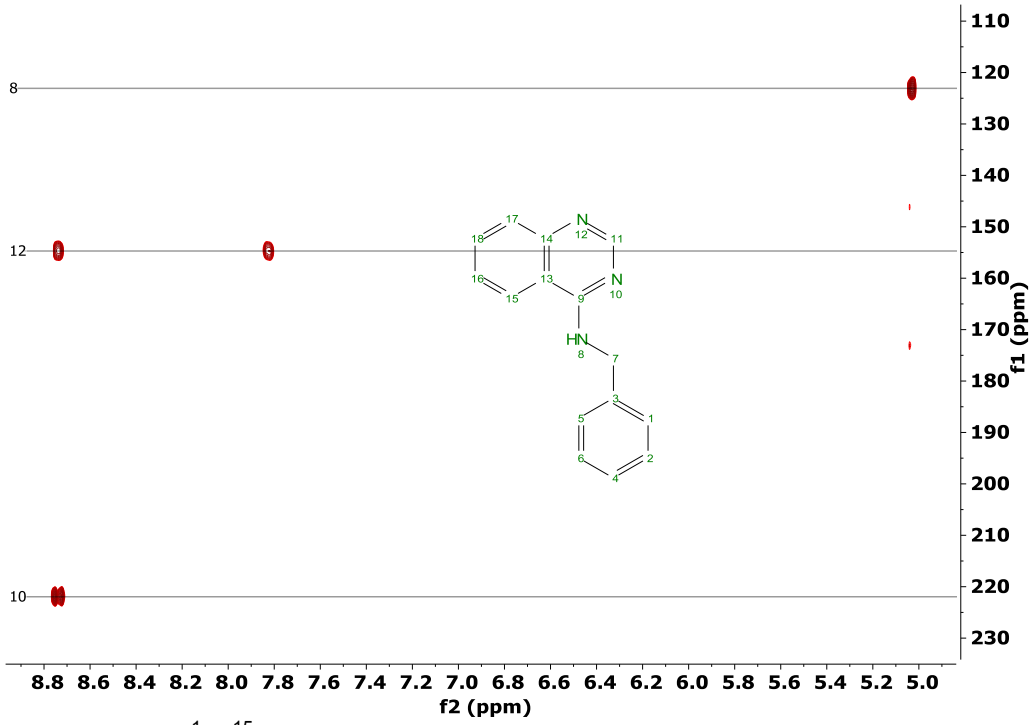


Figure SI 33. A 12 scan ^{1}H-^{15}N HMBC spectrum of SM07 (5.8 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml water-d_2/methanol-d_4 (1:2). The ^{15}N chemical shifts have been assigned by their correlation to near-by protons (no shown). 1.5 equivalents of TFA titrated.

^{1}H-^{15}N HMBC

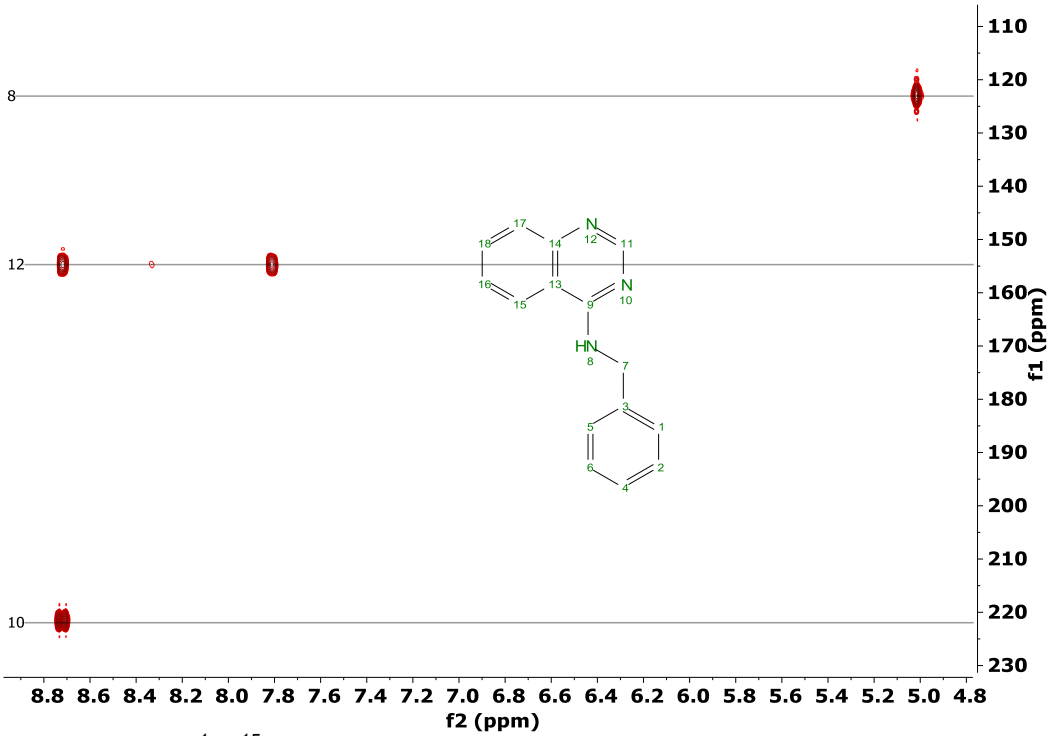


Figure SI 34. A 40 scan ^{1}H-^{15}N HMBC spectrum of SM07 (5.8 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml water-d_2/methanol-d_4 (1:2). The ^{15}N chemical shifts have been assigned by their correlation to near-by protons (not shown). 5 equivalents of TFA titrated.

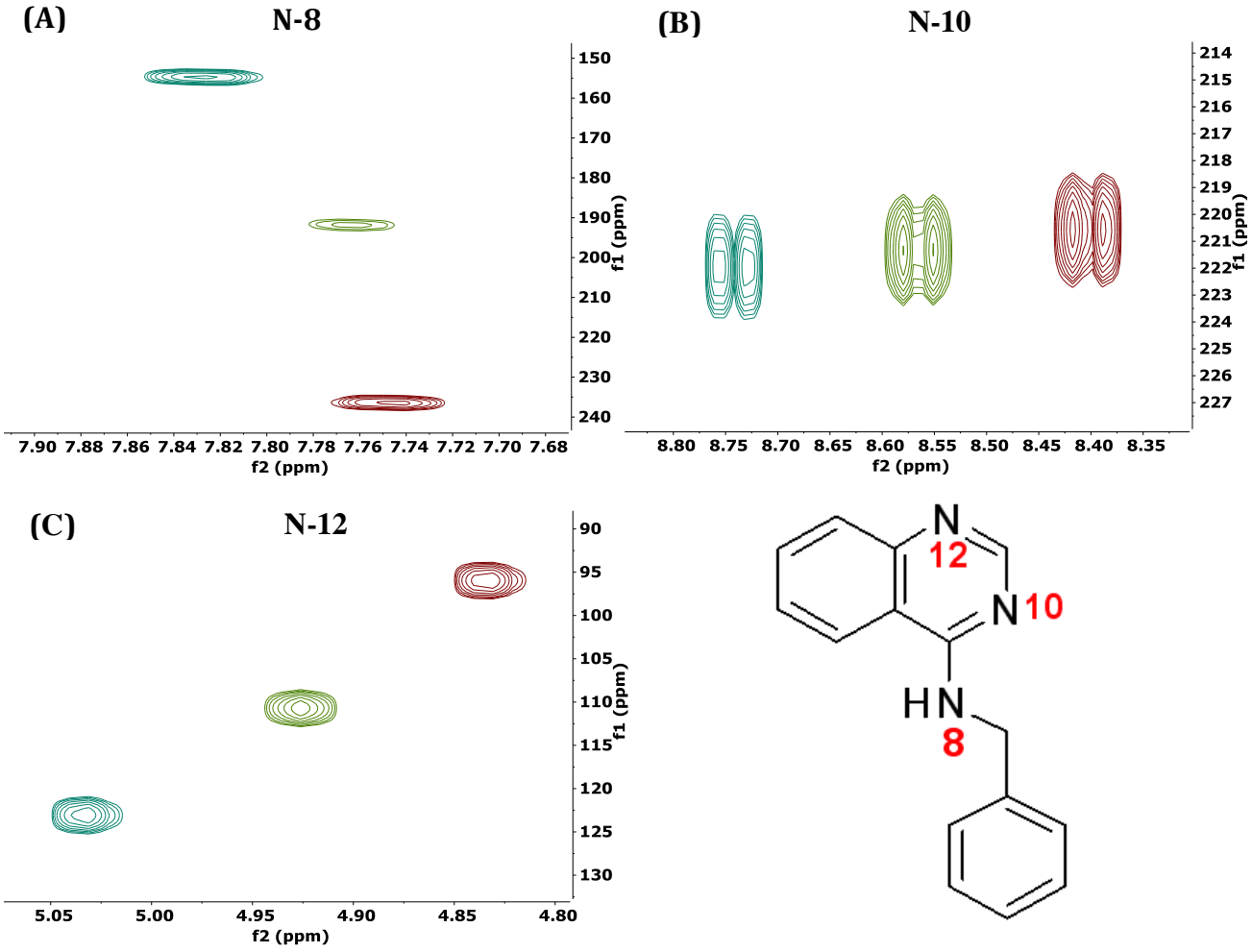


Figure SI 35. Expansions of the overlaid ^{1}H-^{15}N HMBC spectra of SM07 (5.8 mg in 600 µL methanol-d_4/water-d_2, 2:1 v/v ratio) – structure shown – acquired at 298 K on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy after titration with 0.0 (blue), 0.5 (green) and 1.0 (red) equivalents of TFA. Panels (A), (B) and (C) are overlaid cross-peaks of N-8, N-10, and N-12, respectively. No significant chemical shift changes were observed after addition of 1.5 and 5 equivalents of TFA (Fig. SI33 and SI34, respectively).

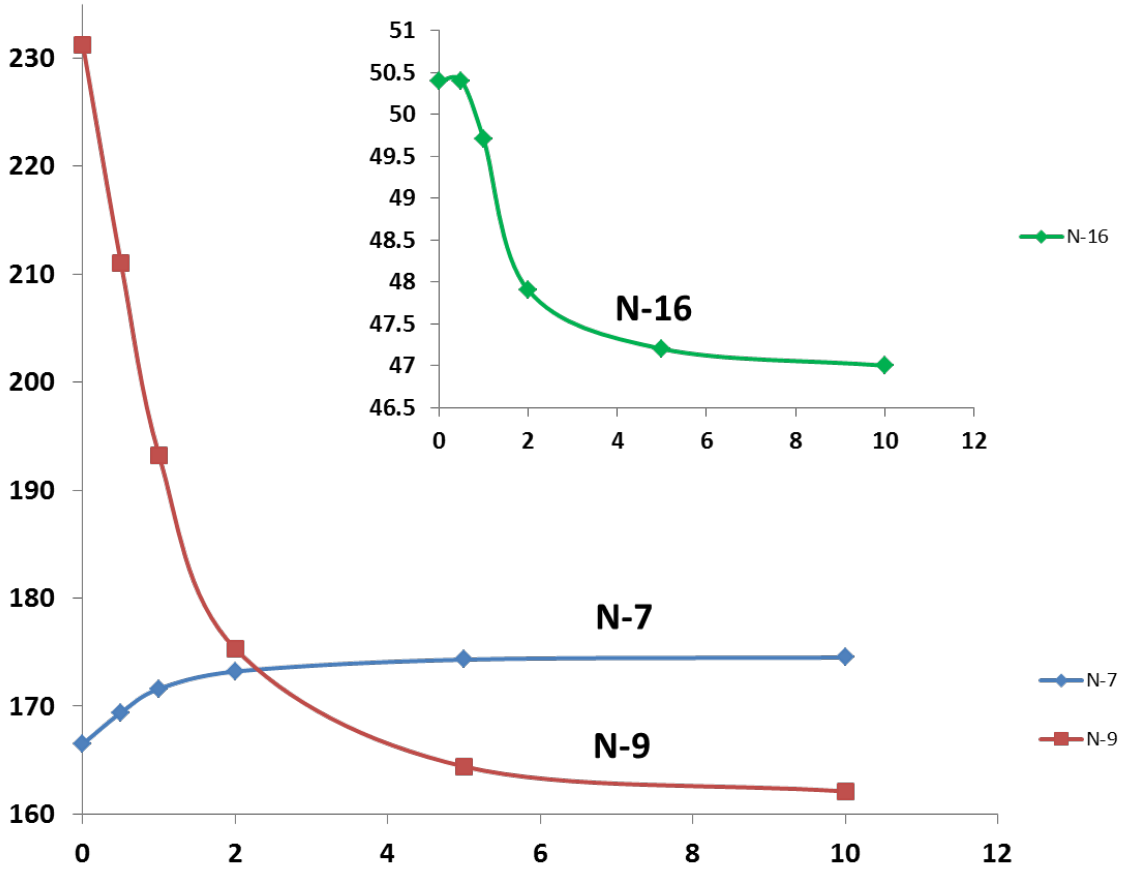


Figure SI 36. Plots of NMR observed chemical shifts of N-7, N-9 and N-16 of SM14 vs. TFA-*d* equivalents titrated in water/methanol (1:2), showing strong solvent effects which complicated the data interpretation. For instance, the plots showed continued protonation of N-9 even after 5 equivalents of TFA-*d*. ^{1}H-^{15}N HMBC experiments (2048 x 256 data points) used for the indirect observation of ^{15}H chemical shifts were acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe^{TM} prodigy at 298 K.

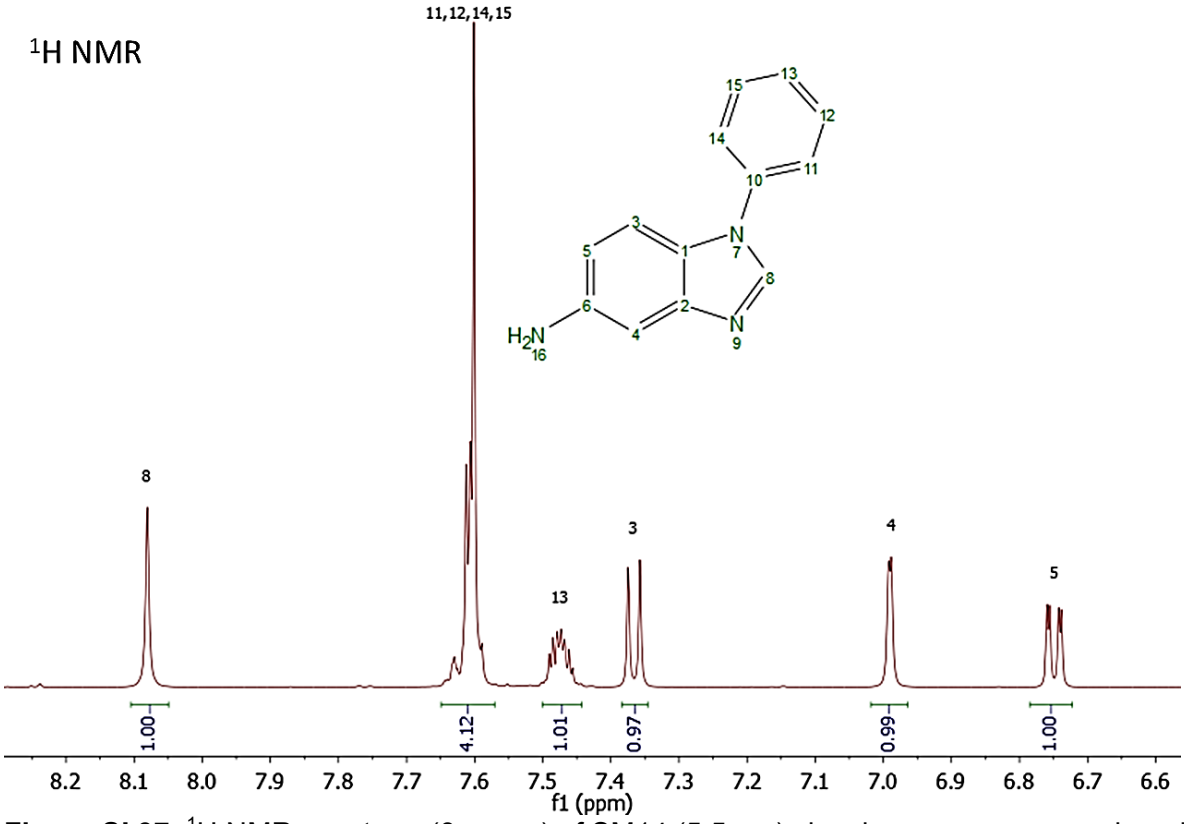


Figure SI 37. ^{1}H NMR spectrum (8 scans) of SM14 (5.5 mg) showing resonances assigned to the corresponding protons. The spectrum was acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml acetonitrile-d_3.

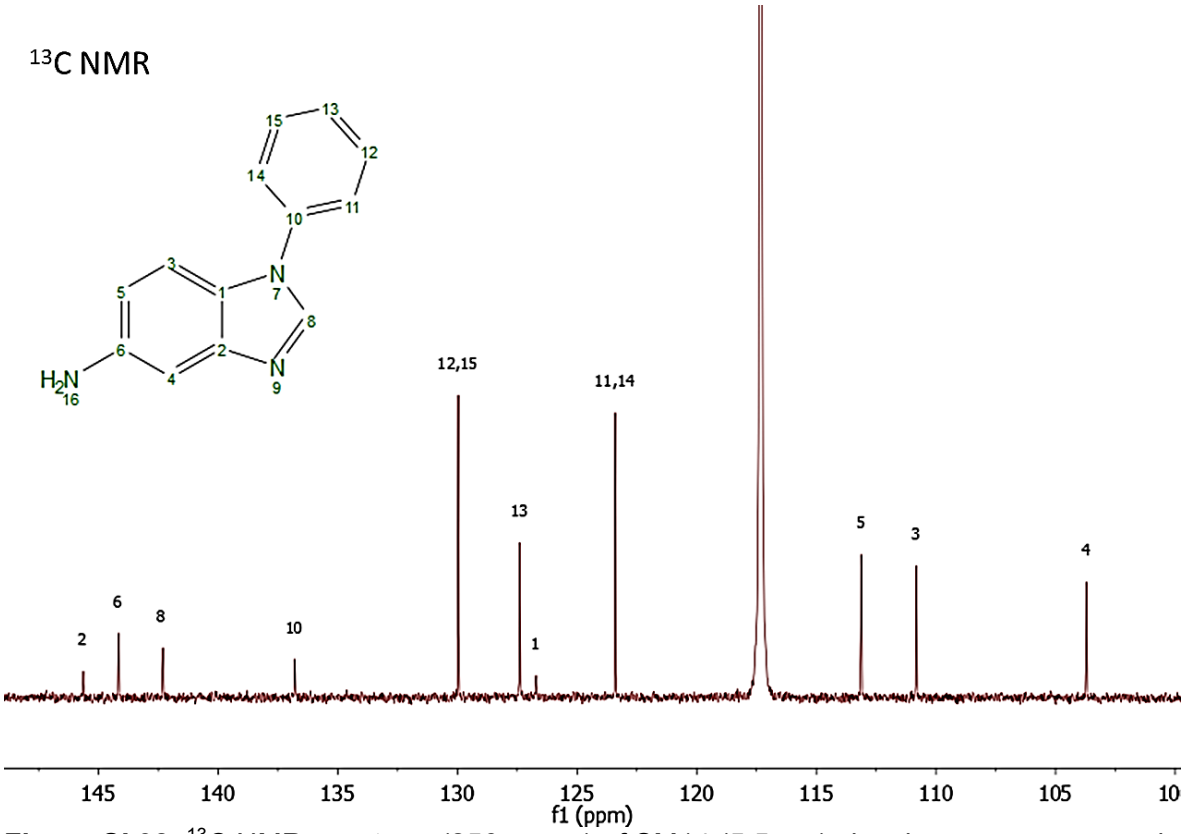


Figure SI 38. ^{13}C NMR spectrum (256 scans) of SM14 (5.5 mg) showing resonances assigned to the corresponding protons. The spectrum was acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml acetonitrile-d_3.

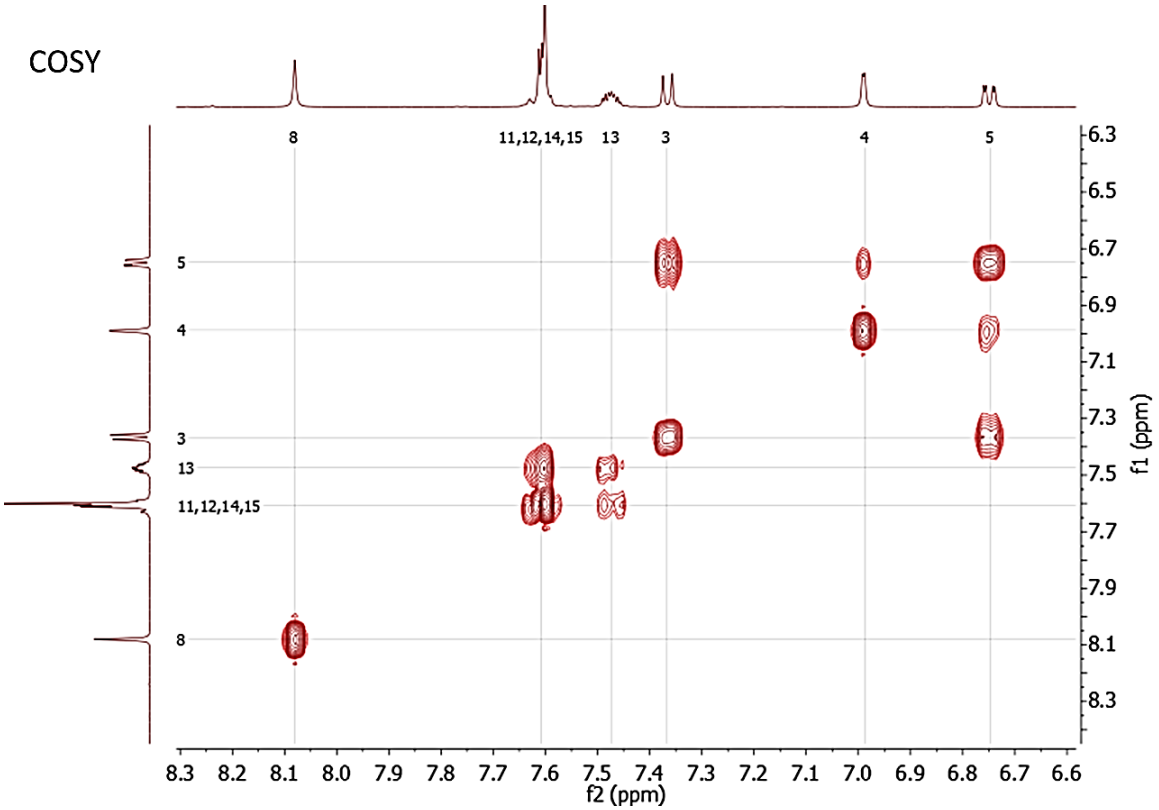


Figure SI 39. A 2 scan COSY spectrum of SM14 (5.5mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml acetonitrile-d_3. Cross-peaks (off-diagonal peaks) corresponds to coupled adjacent protons.

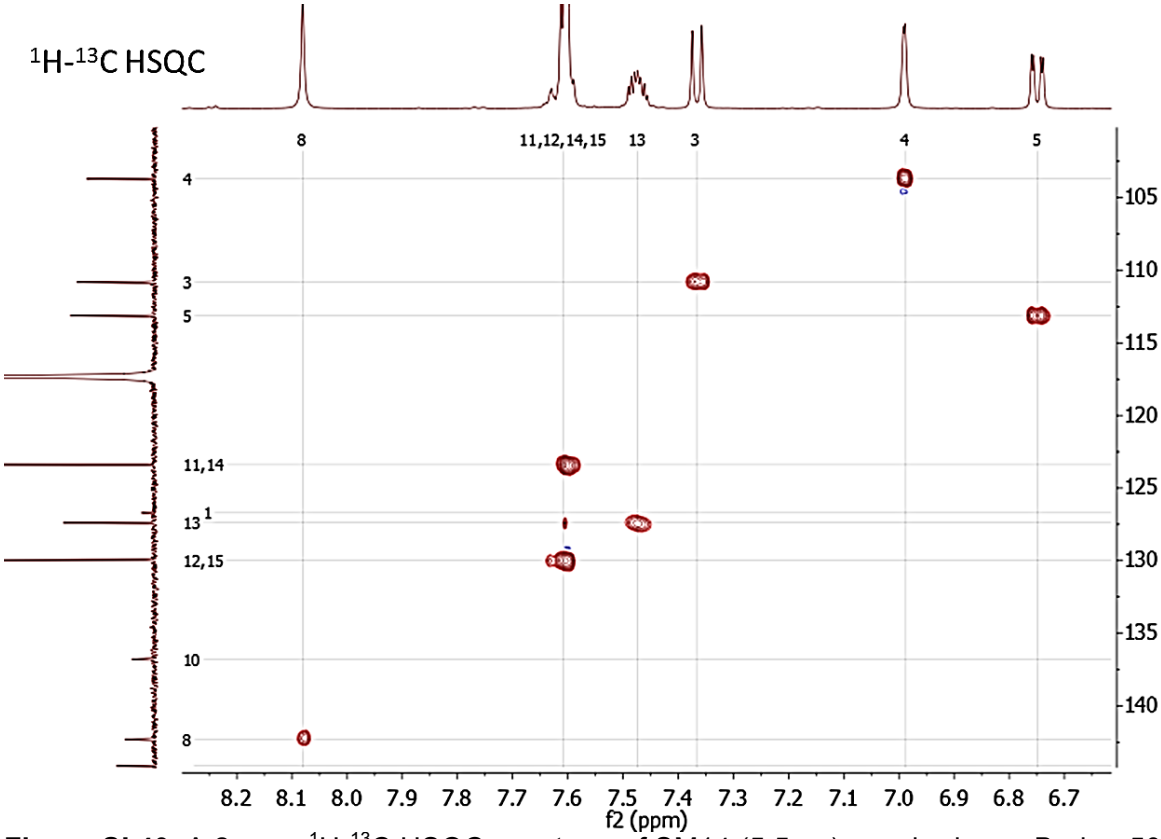


Figure SI 40. A 2 scan ^{1}H-^{13}C HSQC spectrum of SM14 (5.5mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml acetonitrile-d_3. Each peak correlates a carbon to its attached proton(s).

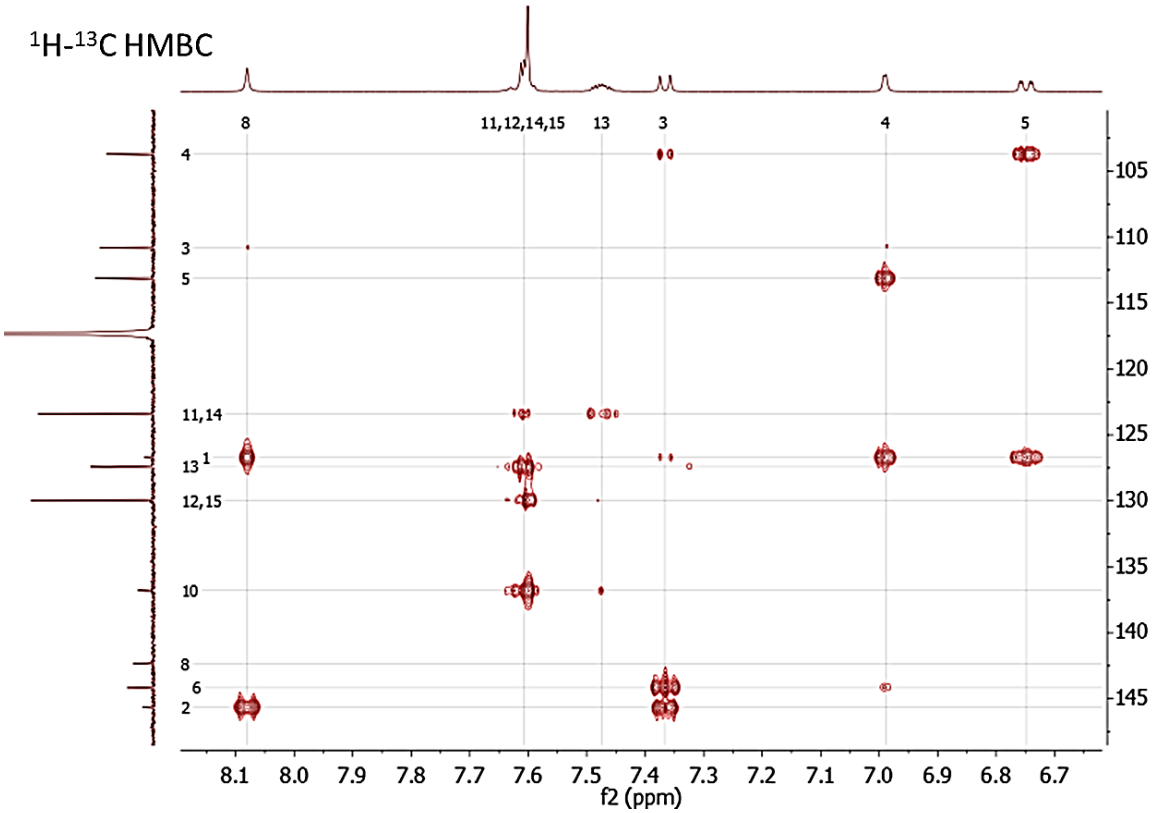


Figure SI 41. A 2 scan ^{1}H-^{13}C HMBC spectrum of SM14 (5.5mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml acetonitrile-d_3. Peaks correspond to coupling between the proton of interest to neighboring nitrogens, which are 2 – 3 bonds away.

^{1}H-^{15}N HMBC

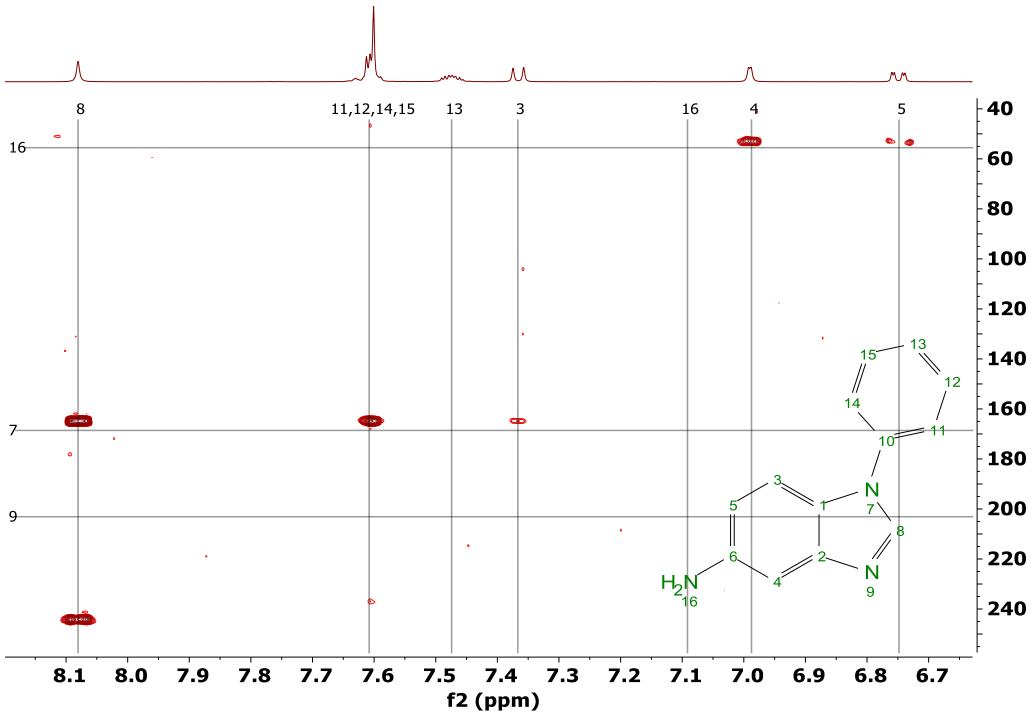


Figure SI 42. A 4 scan ^{1}H-^{15}N HMBC spectrum of SM14 (5.5 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml acetonitrile-d_3. The ^{15}N chemical shifts have been assigned by their correlation to near-by protons. No TFA titrated.

^{1}H-^{15}N HMBC

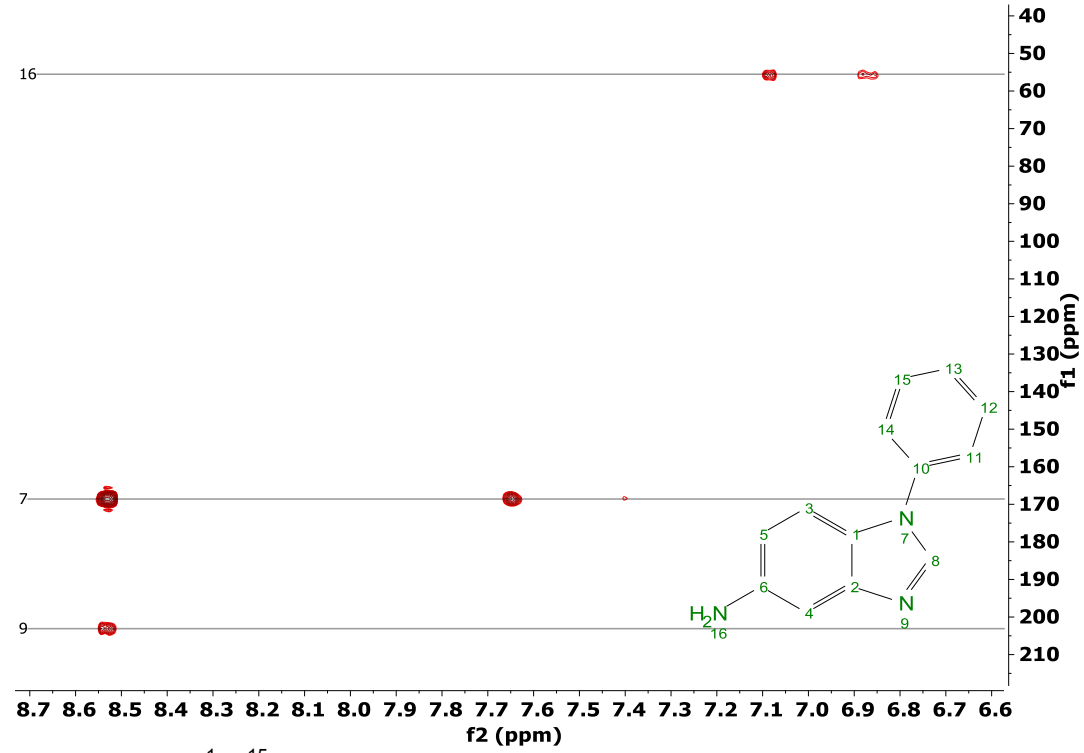


Figure SI 43. A 4 scan ^{1}H-^{15}N HMBC spectrum of SM14 (5.5 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml acetonitrile-d_3 after titration with 0.5 equivalents of TFA. The ^{15}N chemical shifts have been assigned by their correlation to near-by protons (not shown).

^{1}H-^{15}N HMBC

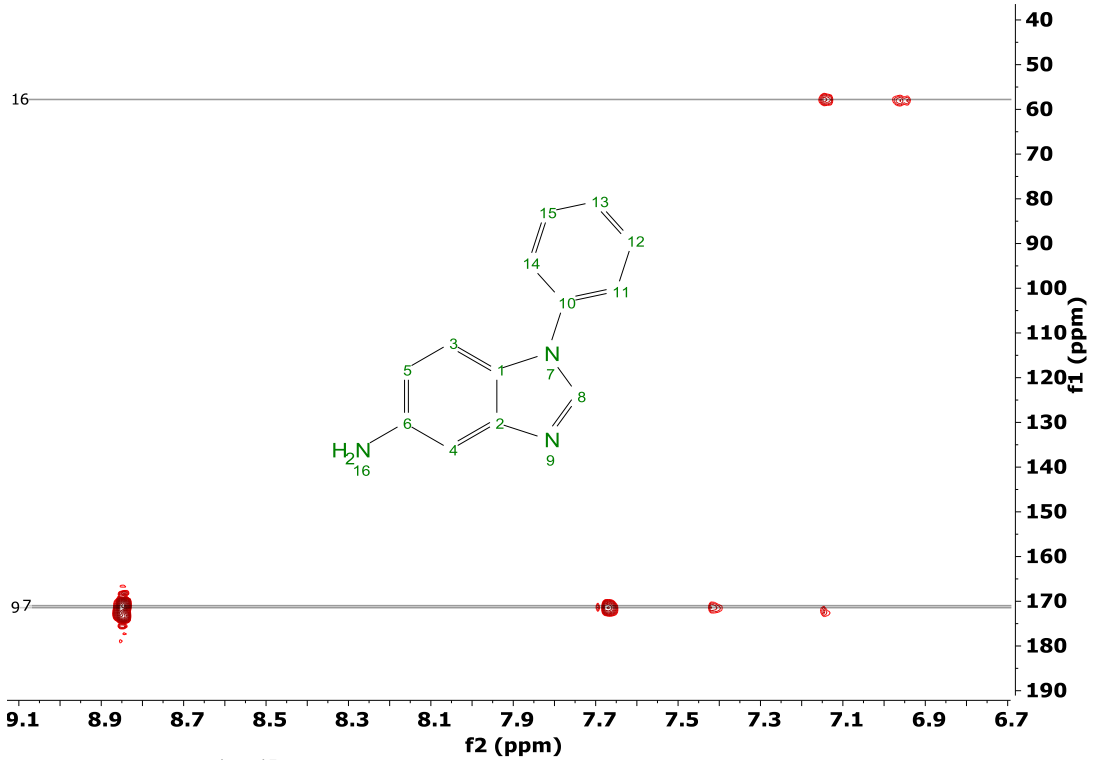


Figure SI 44. A 4 scan ^{1}H-^{15}N HMBC spectrum of SM14 (5.5 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml acetonitrile-d_3 after titration with 1.0 equivalents of TFA. The ^{15}N chemical shifts have been assigned by their correlation to near-by protons (not shown).

^{1}H-^{15}N HMBC

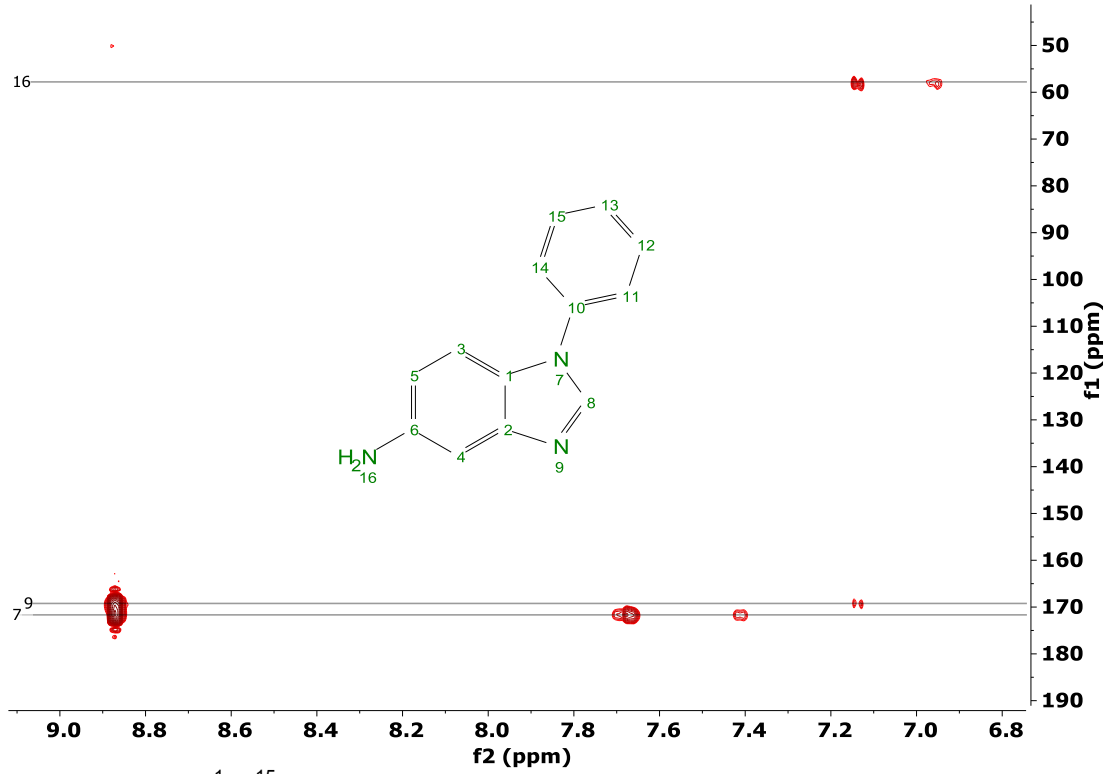


Figure SI 45. A 4 scan ^{1}H-^{15}N HMBC spectrum of SM14 (5.5 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml acetonitrile-d_3 after titration with 1.1 equivalents of TFA. The ^{15}N chemical shifts have been assigned by their correlation to near-by protons (not shown).

^{1}H-^{15}N HMBC

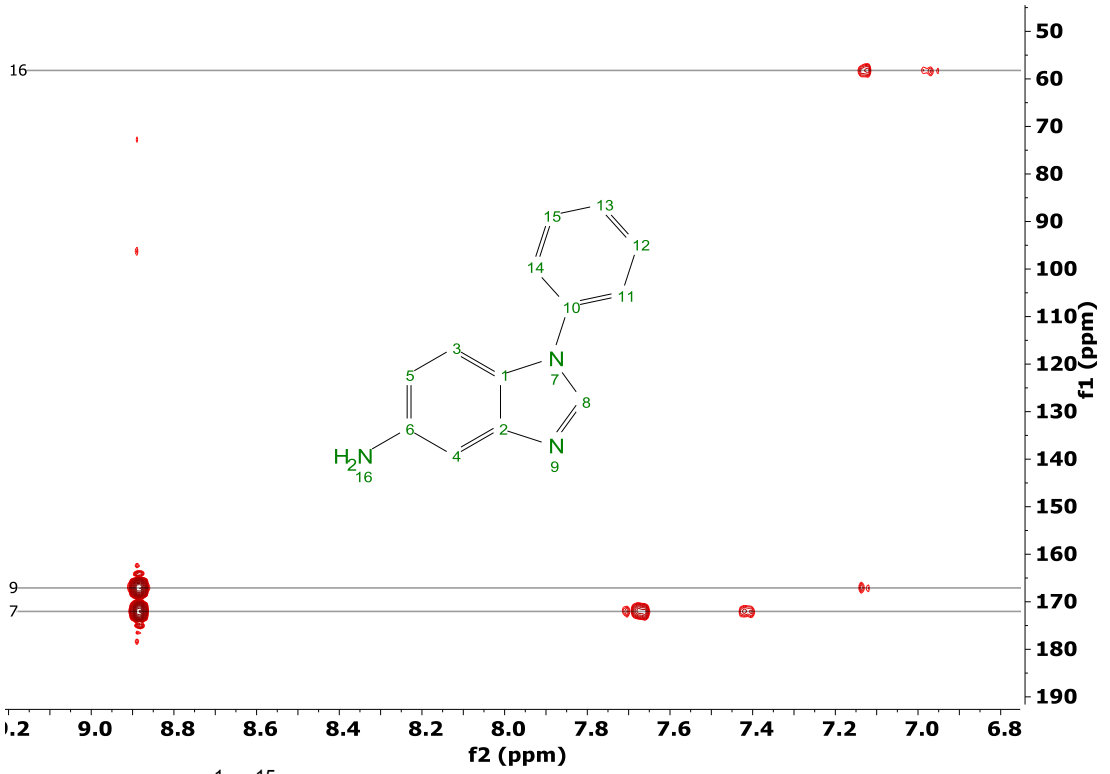


Figure SI 46. A 4 scan ^{1}H-^{15}N HMBC spectrum of SM14 (5.5 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml acetonitrile-d_3 after titration with 1.2 equivalents of TFA. The ^{15}N chemical shifts have been assigned by their correlation to near-by protons (not shown).

^{1}H-^{15}N HMBC

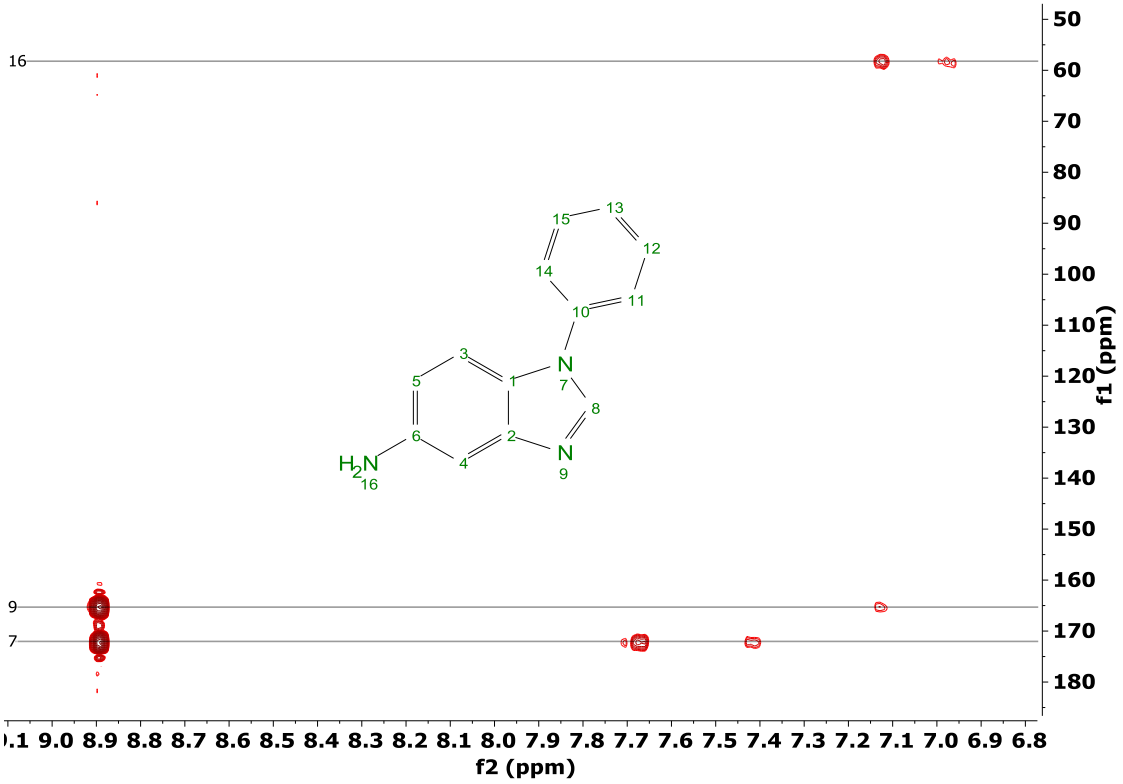


Figure SI 47. A 4 scan ^{1}H-^{15}N HMBC spectrum of SM14 (5.5 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml acetonitrile-d_3 after titration with 1.3 equivalents of TFA. The ^{15}N chemical shifts have been assigned by their correlation to near-by protons (not shown).

^{1}H-^{15}N HMBC

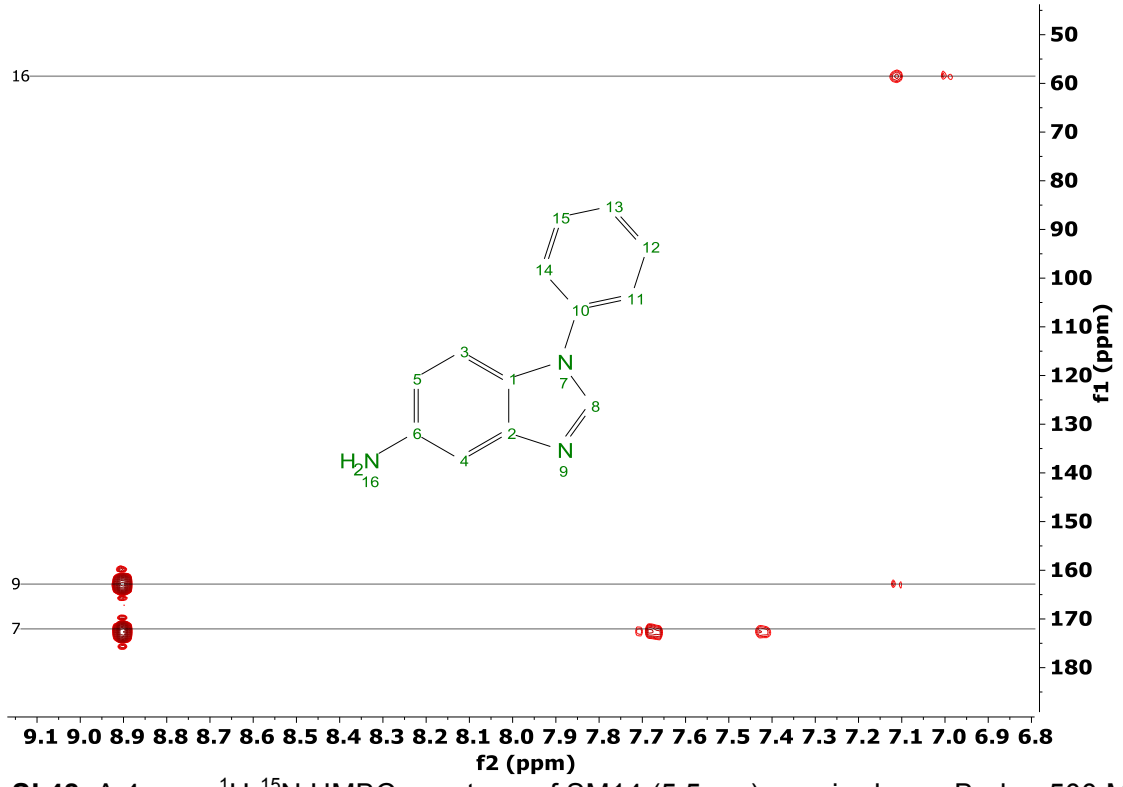


Figure SI 48. A 4 scan ^{1}H-^{15}N HMBC spectrum of SM14 (5.5 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml acetonitrile-d_3 after titration with 1.5 equivalents of TFA. The ^{15}N chemical shifts have been assigned by their correlation to near-by protons (not shown).

^{1}H-^{15}N HMBC

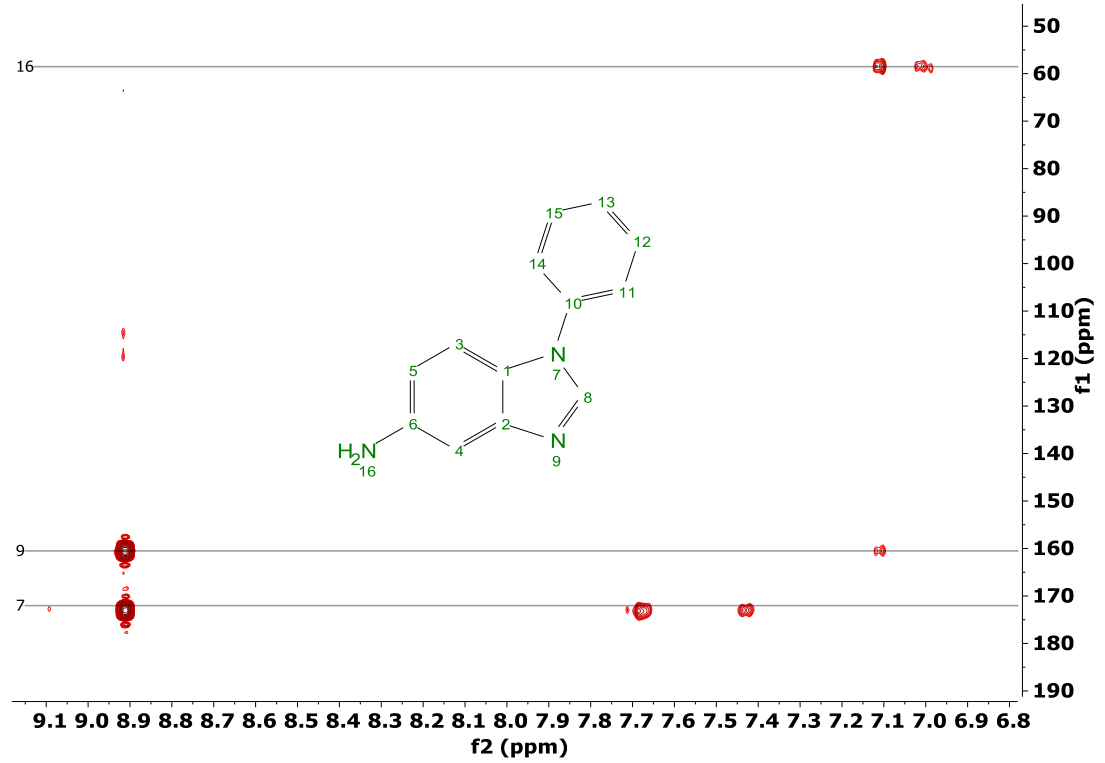


Figure SI 49. A 4 scan ^{1}H-^{15}N HMBC spectrum of SM14 (5.5 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml acetonitrile-d_3 after titration with 1.8 equivalents of TFA. The ^{15}N chemical shifts have been assigned by their correlation to near-by protons (not shown).

^{1}H-^{15}N HMBC

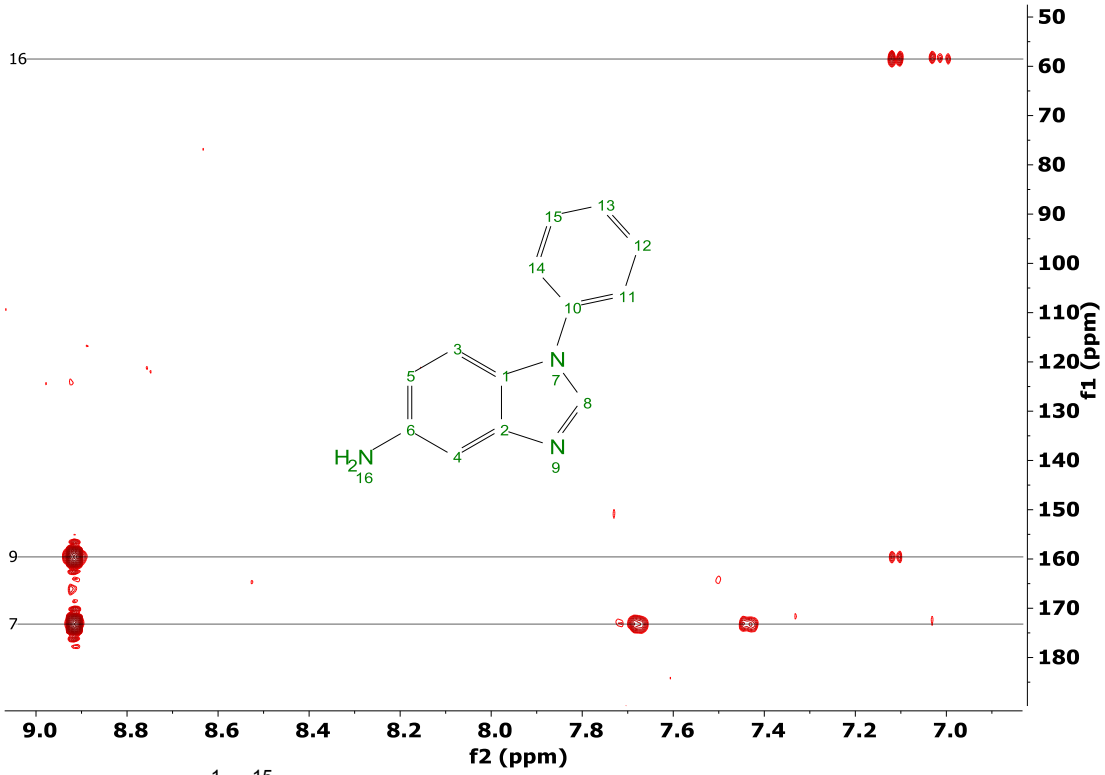


Figure SI 50. A 4 scan ^{1}H-^{15}N HMBC spectrum of SM14 (5.5 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml acetonitrile-d_3 after titration with 2.0 equivalents of TFA. The ^{15}N chemical shifts have been assigned by their correlation to near-by protons (not shown).

^{1}H-^{15}N HMBC

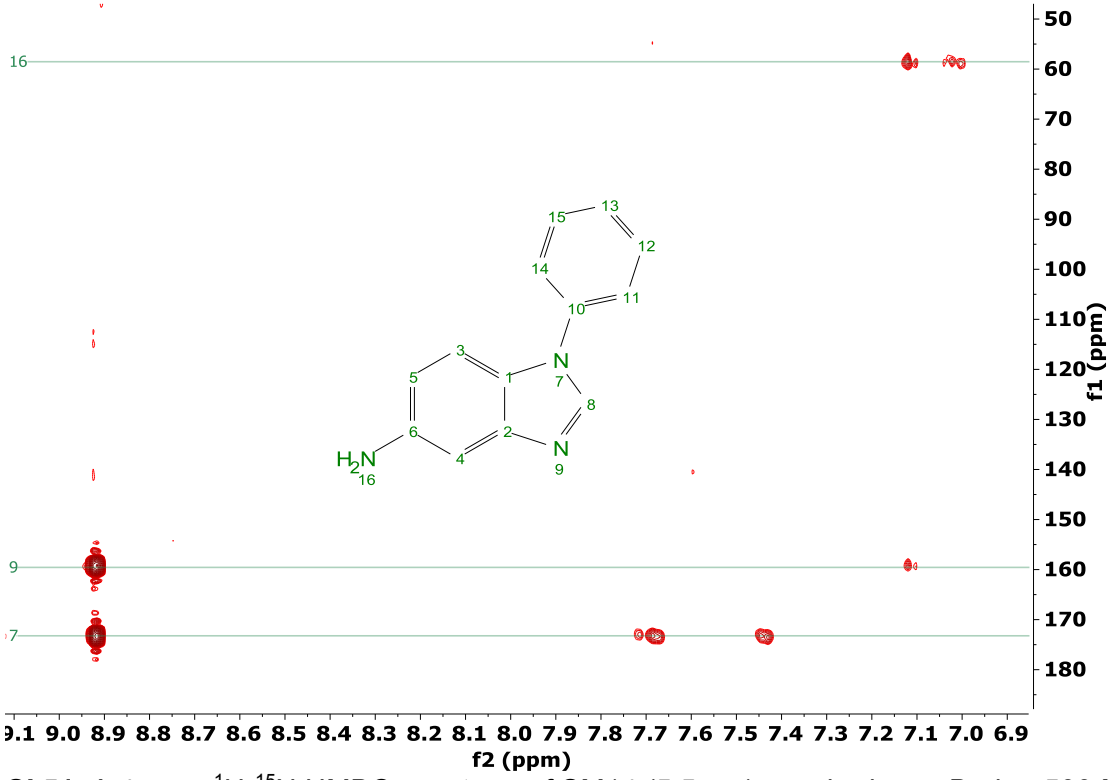


Figure SI 51. A 4 scan ^{1}H-^{15}N HMBC spectrum of SM14 (5.5 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml acetonitrile-d_3 after titration with 2.1 equivalents of TFA. The ^{15}N chemical shifts have been assigned by their correlation to near-by protons (not shown).

^{1}H-^{15}N HMBC

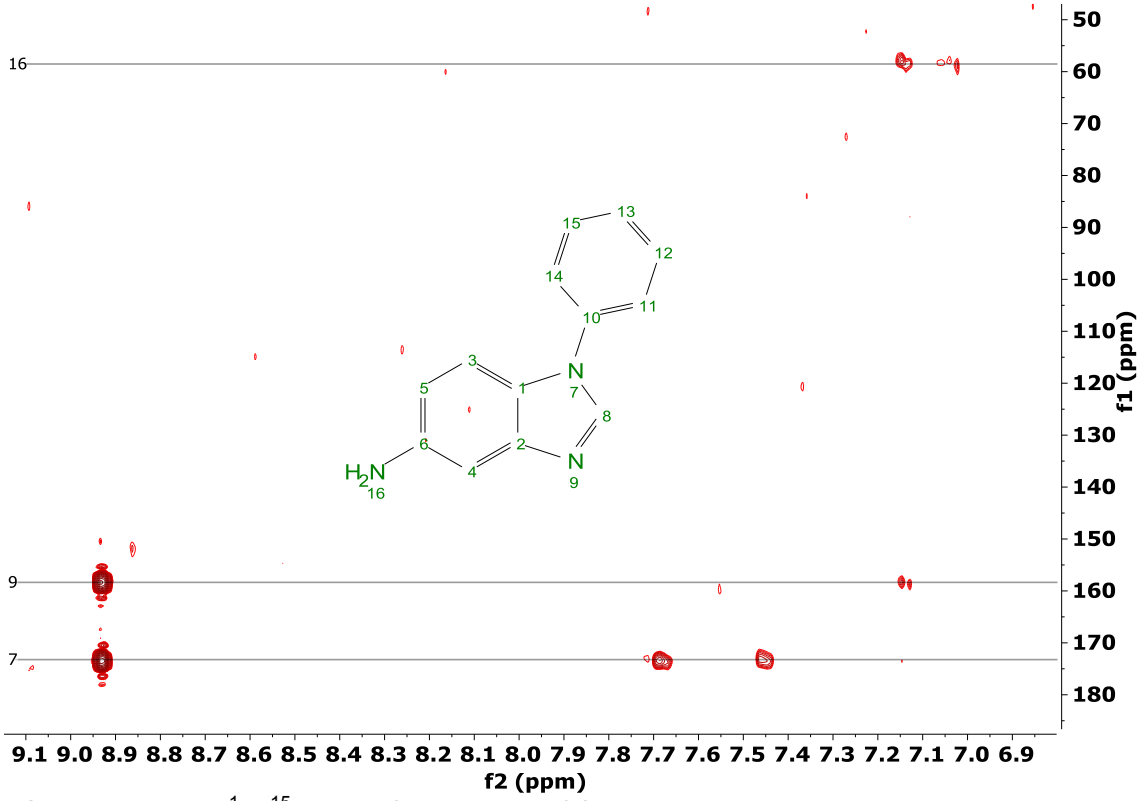


Figure SI 52. A 4 scan ^{1}H-^{15}N HMBC spectrum of SM14 (5.5 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml acetonitrile-d_3 after titration with 2.6 equivalents of TFA. The ^{15}N chemical shifts have been assigned by their correlation to near-by protons (not shown).

^{1}H-^{15}N HMBC

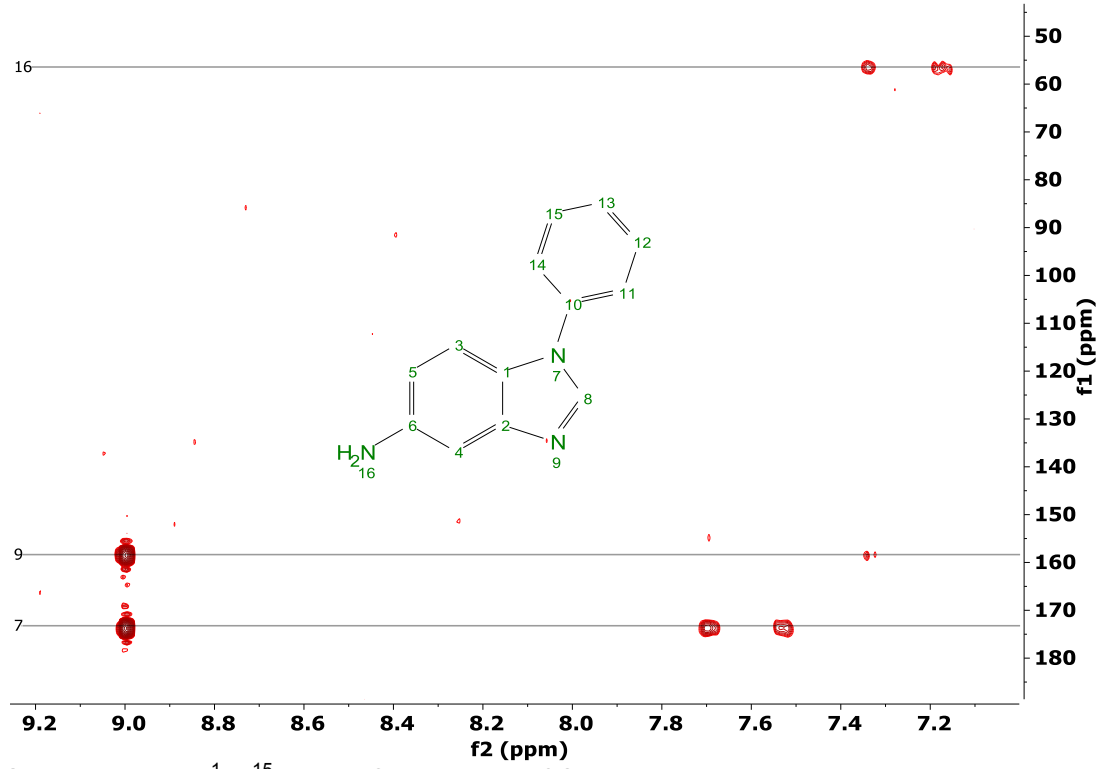


Figure SI 53. A 4 scan ^{1}H-^{15}N HMBC spectrum of SM14 (5.5 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml acetonitrile-d_3 after titration with 5.1 equivalents of TFA. The ^{15}N chemical shifts have been assigned by their correlation to near-by protons (not shown).

^{1}H-^{15}N HMBC

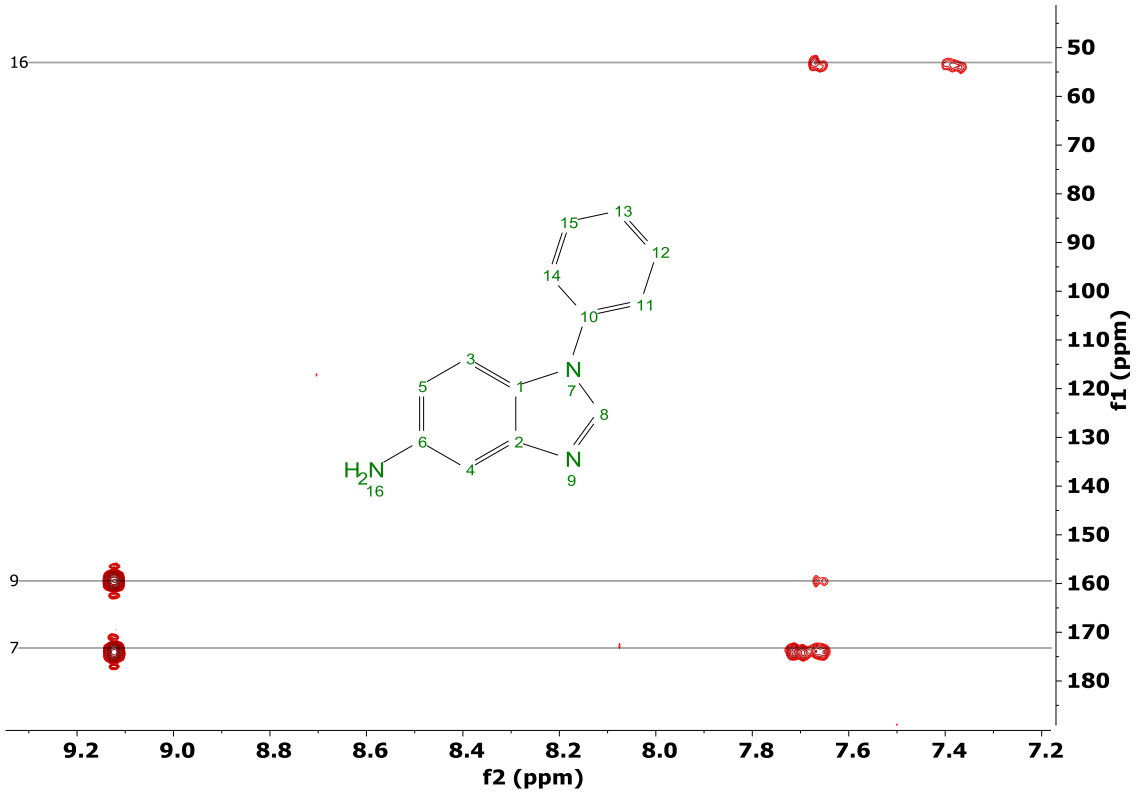


Figure SI 54. An 8 scan ^{1}H-^{15}N HMBC spectrum of SM14 (5.5 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml acetonitrile-d_3 after titration with 10.1 equivalents of TFA. The ^{15}N chemical shifts have been assigned by their correlation to near-by protons (not shown).